PB82-244716

Second US/USSR Symposium: Biological Aspects of Pollutant Effects on Marine Organisms Held at Terskol, USSR on June 4-9, 1979

(U.S.) Corvallis Environmental Research Lab., OR

Apr 82

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Corvallis OR 97333

EPA-600/3-82-034
April 1982

PB82-244716

Research and Development

EPA

Second US/USSR Symposium: Biological Aspects of Pollutant Effects on Marine Organisms

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before compieting)

1. REPORT NO. EPA-600/3-82-034	**2.** ORD Report	**3. RECIPIENT'S ACCESSION NO.** PB 82-244716
4. TITLE AND SUBTITLE Second US/USSR Symposium Biological Aspects of Pollutant Effects on Marine Organisms		**5. REPORT DATE** April 1982 **6. PERFORMING ORGANIZATION CODE**
7. AUTHOR(S) compiled by D.J. Baumgartner and A.I. Simonov		**8. PERFORMING ORGANIZATION REPORT NO.**
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97333		**10. PROGRAM ELEMENT NO.** **11. CONTRACT/GRANT NO.**
12. SPONSORING AGENCY NAME AND ADDRESS same		**13. TYPE OF REPORT AND PERIOD COVERED** symposium proceedings **14. SPONSORING AGENCY CODE** EPA/600/02

15. SUPPLEMENTARY NOTES

Symposium sponsored as part of the US/USSR Agreement on Protection of the Enviornment
Terskol, USSR, June 4 to 9, 1979

16. ABSTRACT

This Symposium was conducted under a US/USSR Environmental Agreement, Project 02.06-21 titled "Effect of Pollutants on Marine Organisms. Papers by American and Soviet specialists present advances in hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on these components. Results of laboratory research as well as field observations on the influence of pollution on the marine environment are presented. Participants attending the Symposium discussed problems related to methods for modeling the influence of pollutants on the marine environment, long term forecasting and determination of permissible loads of pollutants, and the unification and intercalibration of methods for determining biological responses and chemical contamination. Extensive discussions were held on the scope of the technical topics to be included in the exchange. We recognized that EPA's interests were primarily related to the scientific basis for regulating waste discharges, whereas the Soviet scientists tended to be less involved in this activity and more interested in global or at least large scale marine water quality for its own sake. The United States co-chairman proposed to invite greater participation from the National Oceanic and Atmospheric Administration in future project activities to balance the interest of the Soviet scientists. Proceedings are published in English and Russian in compliance with the Memorandum from the 4th Session of the Joint US-USSR Committee on Cooperation in the Field of Environmental Research.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
The authors		

18. DISTRIBUTION STATEMENT Release to public	**19. SECURITY CLASS** *(This Report)* unclassified	**21. NO. OF PAGES** 202
	20. SECURITY CLASS *(This page)* unclassified	**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/3-82-034

SECOND US/USSR SYMPOSIUM
BIOLOGICAL ASPECTS OF POLLUTANT EFFECTS ON MARINE ORGANISMS

Terskol, USSR

June 4 to 9, 1979

Symposium sponsored as part of the US/USSR
Agreement on Protection of the Environment

compiled by

D. J. Baumgartner
Chairman, US Delegation

and

A. I. Simonov
Chairman, USSR Delegation

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON 97333

1.a

DISCLAIMER

This report has been reviewed by the Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

This Symposium was conducted under a US/USSR Environmental Agreement, Project 02.06-21 titled "Effect of Pollutants on Marine Organisms." Papers by American and Soviet specialists present advances in hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on these components. Results of laboratory research as well as field observations on the influence of pollution on the marine environment are presented.

Participants attending the Symposium discussed problems related to methods for modeling the influence of pollutants on the marine environment, long-term forecasting and determination of permissible loads of pollutants, and the unification and intercalibration of methods for determining biological responses and chemical contamination.

The Protocol of the Symposium (the official summary) which follows refers to an introduction to this report. The introductory material to provide background information about technical discussions which took place at the Symposium but which did not result in published reports is included in this abstract.

Extensive discussions were held on the scope of the technical topics to be included in the exchange. We recognized that EPA's interests were primarily related to the scientific basis for regulating waste discharges, whereas the Soviet scientists tended to be less involved in this activity and more interested in global or at least large-scale marine water quality for its own sake. The United States co-chairman proposed to invite greater participation from the National Oceanic and Atmospheric Administration (NOSS) in future project activities to balance the interest of the Soviet scientists. In another way the scope of the exchange was consolidated rather than expanded. This was in relation to the development of two series of symposia -- one for biological effects, the other for "Chemical Pollution of the Marine Environment." There was one proceedings published in the latter series. The participants embraced the concept that biological effects could not be discussed realistically without a consideration of chemical and physical factors, and indeed the papers presented in this biological symposium are not exclusively biological in content. Consequently, it was agreed to abandon the chemical symposium series in favor of one series devoted to an appropriately broad range of scientific disciplines. The next symposium, therefore, to be held in the United States will be labeled simply as the Fourth American-Soviet Symposium on the Effects of Pollution on Marine Organisms.

Proceedings are published in English and Russian in compliance with the Memorandum from the 4th Session of the Joint US-USSR Committee on Cooperation in the Field of Environmental Research.

CONTENTS

Page

Modeling Effects of Environmental Perturbations on Marine Plankton Dynamics 1
Bruce W. Frost

A Quantitative Method for Evaluating External Effects on Ecosystems 15
V. D. Fedorov, V. N. Maksimov and V. B. Sakharov

Petroleum Pollution and Microflora in Marine Ecosystems 26
M. V. Gusev, T. V. Koronelli and V. V. Il'inskiy

Development of Plankton Algae in Conditions of Petroleum Pollution. 43
O. G. Mironov

Long-Term Biological Variability and Stress in Coastal Systems. 52
Robert J. Livingston

Some Features of the Biologic Effects of Pollutants on Marine Organisms . . 67
S. A. Patin

Fish as an Object for Monitoring Petroleum Pollution of the Marine Environment 71
N. D. Mazmanidi, G. I. Kovaleva and A. M. Kotov

Improved Assessment of Ecological Effects by Incorporating Physical Simulations in Bioassay Procedures. 82
D. J. Baumgartner

A Program for Investigating Pollution of Marine Macrobenthos by Heavy Metals and Its Position in the System for Monitoring the Environment. . . . 98
K. S. Burdin and I. B. Savel'yev

Effects of Liquid Industrial Wastes on Estuarine Algae, Plants, Crustaceans, and Fishes 112
Gerald E. Walsh and Richard L. Garnas

Ecologic Aspects of Using Chemical Agents for Eliminating the Results of Oil Spills in the Ocean. 123
M. P. Nesterova

Chemical Pollution of the Film Layer of the Pacific Ocean 131
A. I. Simonov and V. I. Mikhaylov

Preceding page blank

CONTENTS (continued)

Page

Studies on the Biological Transport of Materials From Surface to Deep Ocean Waters: I. Fluxes of Carbon, Nitrogen, and Phosphorus II. Fluxes of Trace Elements. 145
G. A. Knauer and J. H. Martin

Correlation Between Dose Loads in Fish and the Biogeochemistry of Artificial Radionuclides in a Marine Environment 166
I. A. Shekhanova and A. P. Panarin

The Consequences of Chemical Pollution of the "Waterbottom Sediment" Contact Zone in the Sea 180
A. Bronfman and Z. B. Aleksandrova

PARTICIPANTS

American Side

Dr. Donald J. Baumgartner
Co-chairman of the Symposium
Environmental Research Laboratory
U.S. Environmental Protection Agency
Corvallis, Oregon

Dr. Bruce W. Frost
Department of Oceanography
University of Washington
Seattle, Washington

Dr. George A. Knauer
Moss Landing Marine Laboratories
Moss Landing, California

Dr. Robert J. Livingston
Department of Biological Science
Florida State University
Tallahassee, Florida

Dr. Gerald E. Walsh
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida

Soviet Side

Dr. Anatoliy I. Simonov
Co-chairman of the Symposium
State Oceanographic Institute
Moscow

M. P. Nesterova
Deputy Co-chairman of the Symposium
P.P. Shershov Oceanology Institute
USSR Academy of Sciences

Dr. Vadim D. Fedorov
Head of the Chair of Hydrobiology
Moscow State University

Dr. Mikhail V. Gusev
Dean, Department of Biology
Moscow State University

O. G. Mironov
Institute of Biology
for Southern Seas
AN USSR, Sevastopol'

S. A. Patin
All-Union Fisheries and Oceanograpy
Scientific Research Institute
Moscow

N. D. Mazmanidi
Georgian Branch of the VNIRO

K. S. Burdin, Docent
Department of Biology
Moscow State University

V. I. Mikhaylov
State Oceanographic Institute
Odessa Branch

Irina A. Shekhanova
Head of the Radiation
Biology Laboratory
All-Union Scientific-Research
Institute of Marine Fisheries
and Oceanography
Moscow

A. I. Bronfman
Economics Institute of the
Ukrainian Academy of Sciences
Odessa

Dr. Ralfrid A. Gasanov
Institute of Botany
Academy of Sciences of
Azerbaijan S.S.R.
Baku

Dr. Gasanov presented a paper entitled "Biophysical Parameters of Photosynthesizing Organisms in a System for Monitoring Marine Pollution." His co-authors on the paper were N. M. Aliyev, Z. Sh. Aliyev, and N. M. Karayeva. The paper was not available for publication in this volume of the proceedings.

PROTOCOL OF THE SECOND US/USSR SYMPOSIUM

BIOLOGICAL ASPECTS OF POLLUTANT EFFECTS ON MARINE ORGANISMS

In accordance with the principles outlined in the Memorandum of the VII Session of the Joint US/USSR Committee Cooperation in the Field of Environmental Protection (January 29 - February 2, 1971, Moscow, USSR), the Second US/USSR Symposium on Biological Aspects of Pollutant Effects on Marine Organisms was held in settlement Terskol, June 4-9, 1979.

Dr. D. J. Baumgartner, Director, Marine Division, Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, and Prof. A. I. Simonov, Chief of Department of the State Oceanographic Institute (USSR), were co-chairmen of the Symposium.

The participants of the Symposium presented reports on modeling the effects of pollutants on ecological systems, marine plankton dynamics influenced by natural and anthropogenic factors, microflora and planktonic algae of marine ecosystems containing petroleum hydrocarbons, marine toxicological bioassays, the use of models to incorporate physical conditions in bioassays, relationships of dose loads in fish with radionuclide biogeochemistry in the marine water, ecological aspects of utilization of chemical means for the elimination of oil spills in the sea, oceanic surface layer chemical pollution, significance of chemical interactions in the seawater-seabed interface, biological consequences of marine environmental chemical pollution including effects on marine macrobenthos pollution by heavy metals, fish contamination by petroleum hydrocarbons, and application of biophysical parameters of photosynthesizing organisms while monitoring.

The participants expressed their wish to hold the Third Symposium on biological aspects of pollution effects on marine organisms and marine pollution dynamics in 1-2 years in the United States after conducting joint and independent studies. Problems concerned with estuaries and the continental shelf in the selected regions of the world's oceans would receive attention in accordance with the program of long-term research designed at the joint US/USSR meeting in September 1978 (Gulf Breeze, Moss Landing, Corvallis, USA). The number of participants of the USSR delegation to the Symposium, date and place will be agreed upon by Co-chairmen of the Project by correspondence by October 1979 and submitted for the consideration to the VII Session of the US/USSR Joint Committee on Cooperation in the Field of Environmental Protection.

Plans were made for the next step in metal intercalibration studies and a schedule was developed for two USSR scientists to visit the EPA marine research laboratory at Narragansett, Rhode Island. This 2-week working visit will begin July 1, 1979 for the purpose of exchanging information on methods to analyze oil pollutants in seawater, sediments, and marine animal tissues.

Both sides agreed to publish the Symposium proceedings during 1980. Co-chairmen agreed to write the introduction to the proceedings of the Second Symposium by correspondence by October 1, 1979. They also agreed to send to each side final texts of reports by July 1979. The USSR side will present its reports in English and the United States reports will be presented in Russian.

Both sides consider it important to study biological effects of chemical pollution in estuaries as well as to develop techniques of investigations and design suitable predictive models. While developing techniques, one should proceed both from the purposes and tasks of research. Apart from the tasks, however, the sides believe it is significant to use known hydrodynamic models to carry out complex observations that include both abiotic and biotic components. The principal factors that should be included are those that influence the ionic form of pollutants in rivers and those responsible for precipitation, dissolution, and ionic speciation in seawater (salinity and other chemical properties of seawater). Each specific estuary is likely to have a characteristic species composition which may dictate the factors that need to be included in a measurement program.

If the task is to study long-term changes of hydrobiological conditions, then multiyear observations should be performed, similar to those demonstrated in Dr. Livingston's paper, with subsequent statistical processing with the help of systems analysis. If the task is to track the short-term changes due to pollutant effects, then limited but systematic observations are required when suitable biological monitors are selected, as it was shown in the paper by Dr. K. Burdin and Dr. Savel'jev. In developing models to describe hydrobiological condition changes caused by both natural and anthropogenic factors, long-term observations should be combined with experimental laboratory data.

Both sides agreed that, in field assessments, it is recommended that samples are to be taken both for chemical and biological analysis in the surface water microlayer, thermocline layer, near bottom horizons, and in bottom sediments, similar to procedures proposed in papers by Drs. A. Simonov and V. Mikaylov; G. Knauer and J. Martin; M. Gusev, T. Koronelli and V. Il'inskiy. Both sides consider it reasonable to conduct investigations in coastal estuaries both in the USA and USSR and exchange results of investigations. The estuaries for study, as well as methods for exchanging and discussing the investigation results, will be agreed upon by Co-chairmen by correspondence before the end of 1979. Research on the continued improvement of bioassays, as discussed by Drs. Walsh and Baumgartner, was considered in relationship to the importance of protecting estuarine and coastal ecosystems from pollution stress.

During the visit to the USSR, the American delegation visited Elbrus scientific station of the Geographical Department of the Moscow State University (settlement Terskol), Georgian All-Union Scientific-Research Institute of Fishery and Oceanography in Batumi and the Biological Department of the Tbilissi and Moscow State Universities.

The Symposium was held in the spirit of friendship, cooperation, and mutual benefit. The United States delegation expresses gratitude to the Soviet side for the excellent organization of the Symposium and for their generous hospitality. The delegation thanks interpreters for their excellent work.

The protocol was signed in settlement Terskol, on June 9, 1979 in English and Russian. Both copies are equally valid.

D. J. Baumgartner Chairman of the Project from the US side	A. I. Simonov Chairman of the Project from the USSR side

MODELING EFFECTS OF ENVIRONMENTAL PERTURBATIONS ON MARINE PLANKTON DYNAMICS

by

Bruce W. Frost
Department of Oceanography
University of Washington
Seattle, Washington

INTRODUCTION

Assemblages of marine planktonic organisms with varying degrees of trophic complexity have been experimentally treated with pollutants such as heavy metals, chlorinated hydrocarbons, and petroleum hydrocarbons. It has been repeatedly observed that, because species differ in their tolerance to pollutants, the major effect on assemblages is a shift in the species composition of one or more trophic types, often without concomitant effects on standing stock or rate measurements (Menzel 1977). Therefore, simulation models which attempt to represent the effects of pollutants on plankton dynamics should describe the structure of the plankton as well as biomass and production.

One such model of plankton dynamics was recently developed by Steele and Frost (1977). The model simulates the structure and dynamics of phytoplankton and herbivorous zooplankton during and after a spring bloom in a temperate ocean. In the theoretical study described below, a reasonably realistic simulation is characterized, then the model ecosystem is perturbed in a variety of ways to simulate some effects of pollutants which have been documented in the literature.

METHODS

A revised version of the simulation model of Steele and Frost (1977) was used to examine some potential effects of pollutants on the structure and dynamics of marine plankton communities. Briefly, the model simulates growth of 20 size classes of phytoplankton and the population dynamics of two species of filter-feeding copepods.

Except for change in the value of one parameter, the submodel specifying phytoplankton growth remains as described by Steele and Frost (1977). That is, growth of size classes of phytoplankton is determined by size-dependent functions of nutrient uptake, respiration, sinking, and grazing. Variations in size-specific patterns of growth of the phytoplankton were produced by changing the value of the parameter a in the equation

$$R = aD^{-1/3} \qquad (1)$$

where R is specific respiration rate (mg C/mg plant C per day) and D is diameter (μm) of a phytoplankton cell. The parameter a was 0.36 in the standard simulation.

Substantial revisions were made in the submodel of population growth of planktonic grazers. The two species of grazers in the model are planktonic copepods similar to *Calanus pacificus* (a close relative of *C. helgolandicus*) and *Pseudocalanus* sp. These species have fundamentally different patterns of optimal growth (Frost 1979). Therefore, separate submodels were used for the two grazer species. All parameters of population growth for the two species are identical to those used by Steele and Frost (1977) except the maximum specific growth rate, G_{max}, which in the standard simulation takes the value of 0.4 for *Calanus* and 0.2 for *Pseudocalanus*. The rationale for this change is discussed by Frost (1979).

A second major change concerns the procedure of Steele and Frost (1977) for describing the growth of copepods as passage of individuals through discrete size (weight) classes. This procedure causes a severe problem of "numerical dispersion" (Evans *et al*. 1977) and was therefore replaced by a multiple cohort reproduction scheme (see Landry 1976) in which each day's reproductive products are followed as a cohort until all members of the cohort die. This gives a more realistic pattern of growth in the copepods.

Finally, the description of predation rate on the zooplankton species was greatly simplified from that used by Steele and Frost (1977). It is assumed that predation rate depends only on abundance of a grazer species

$$\text{Predation Rate} = \frac{mZ}{H + Z} \tag{2}$$

where m is the maximum predation rate (% per day), Z is population size (numbers per m^2) of a grazer species, and H is the population size at which predation rate is half the maximum rate. A specific population of predators is not simulated. The population dynamics of the two grazer species are described separately in the model and the parameter H takes the value 100,000 per m^2 for *Calanus* and 200,000 per m^2 for *Pseudocalanus*. This is equivalent to assuming that the two zooplankton species have different predators. The parameter m is 0.1 in the standard simulation.

In all other respects, the format and parameter values of the simulation model are identical to those described by Steele and Frost (1977).

RESULTS

Standard Simulation

A reasonably realistic simulation, the standard simulation, was selected through trial and error by varying the coefficient for algal respiration, a (eq. 1). The criterion for realism was that both *Calanus* and *Pseudocalanus* maintained breeding populations for 100 days.

The standard simulation (Figure 1A) predicts changes in concentration of nitrate and standing stock of phytoplankton which are quite similar to those of the basic run of Steele and Frost (1977: Figure 17). The major difference is the relative abundance of the species of copepods (Figure 1B). In this revised model, Calanus dominates the zooplankton biomass during and after the phytoplankton bloom, primarily because of its higher maximum specific growth rate.

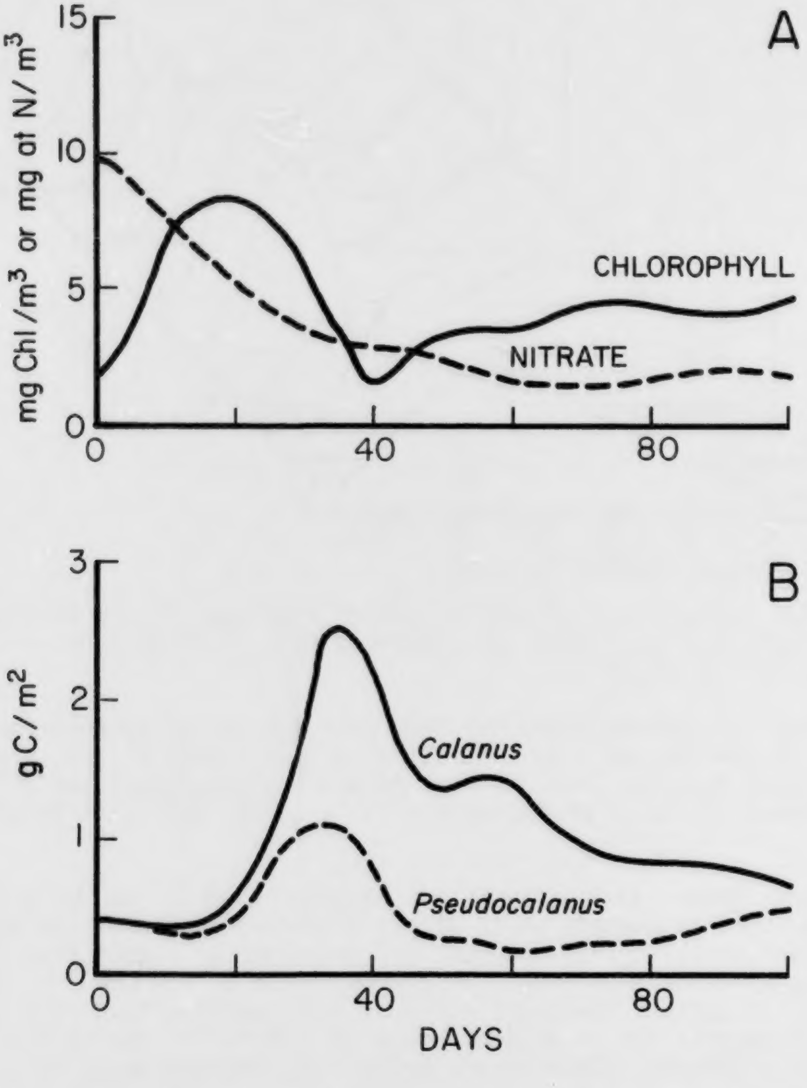


Figure 1. Simulated dynamics of plankton during and after a spring bloom in a temperate ocean. This is the standard simulation. A, changes in time of chlorophyll and nitrate. B, changes in time of biomass of populations of Calanus and Pseudocalanus.

The phytoplankton size composition (Figure 2) is strongly affected by grazing and develops a bimodal distribution during the last 50 days of the simulation. The larger mode in Figure 2 represents the sizes of cells with optimum intrinsic growth rates at the ambient nitrate concentrations, while the smaller mode represents cells which have low intrinsic growth rates but are also very inefficiently utilized by grazers.

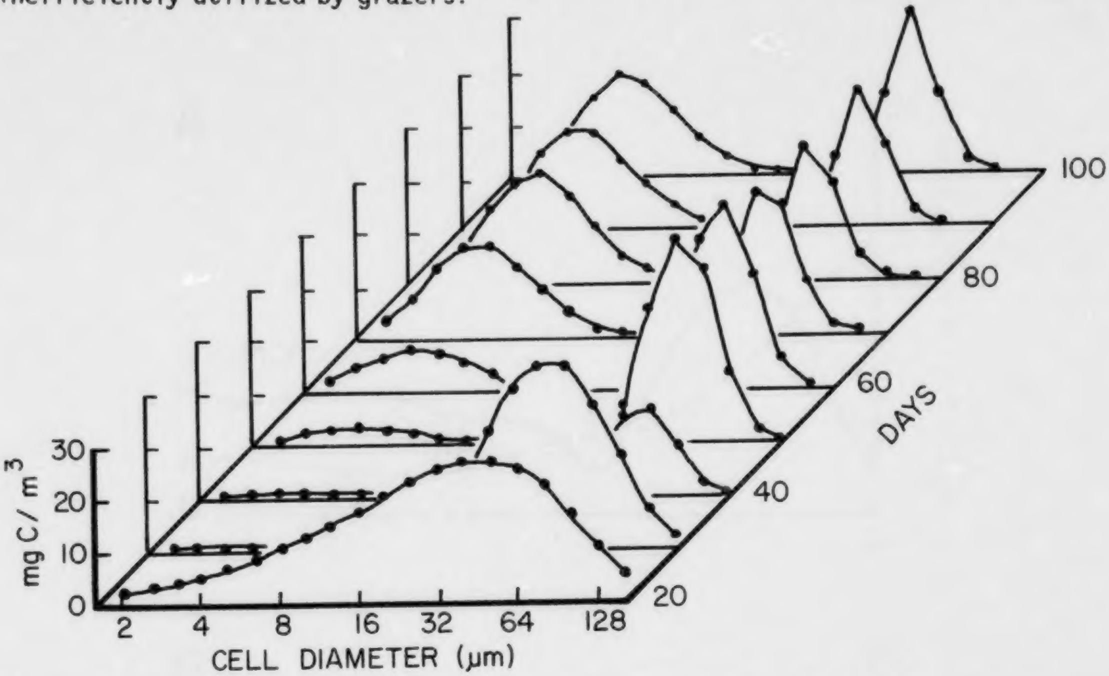


Figure 2. Size composition of phytoplankton from day 20 to day 100 in the standard simulation. Each point gives the concentration in a discrete size category.

Population structures indicate that both species of copepods produce two generations in the 100-day simulation (Figure 3). Adults of the first generation of Calanus recruit about 15 days before Pseudocalanus, which is due to the predominance of large phytoplankton cells during the first 60 days of the simulation.

Some very generalized effects of pollutants will now be examined by altering, in turn, parameters which affect size composition of the phytoplankton, growth and mortality in copepods, and abundance of higher trophic levels. The simulated perturbations could, of course, occur at any time in the seasonal cycle, yet to illustrate the basic patterns it is assumed that induced changes in parameters persist for an entire simulation. Moreover, possible adaptations of species to pollutants (Stockner and Antia 1976) are ignored.

Changes in Size Composition of Phytoplankton

A variety of pollutants cause shifts in the structure of the phytoplankton, generally toward predominance by very small cells (Menzel 1977;

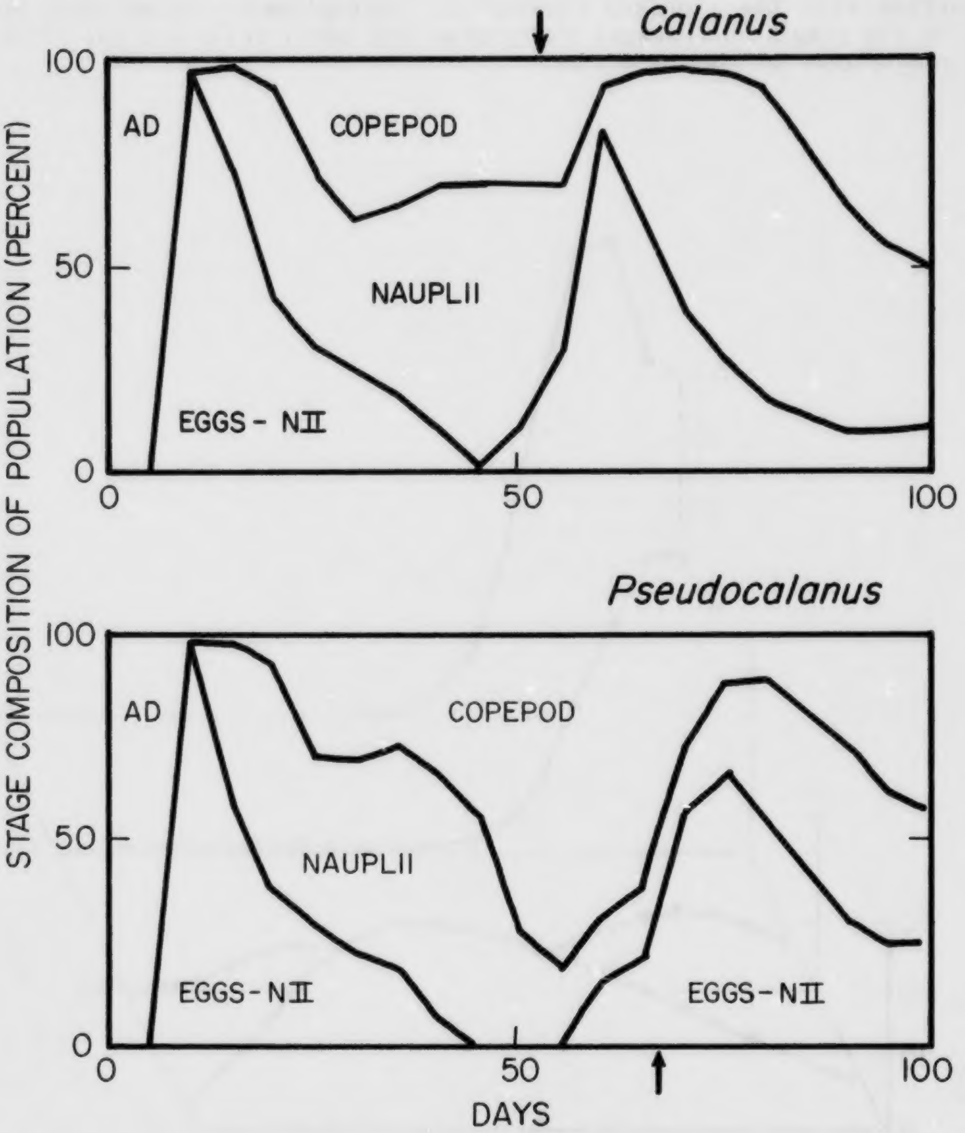


Figure 3. Percentage composition of populations of Calanus and Pseudocalanus in the standard simulation. After day 15 the category copepods includes adults (ad). The category nauplii includes naupliar stages III-VI. Arrows indicate time of recruitment of adults of the first generation.

Greve and Parsons 1977). This effect may be simulated by changing the value of the parameter a (eq. 1) which specifies the size dependence of respiration. When the paramenter is decreased from 0.36 to 0.24, the phytoplankton size composition rapidly shifts toward small cells (Figure 4). The cycle of standing stock of the phytoplankton (Figure 5A) is also severely disrupted in comparison with the standard simulation. Phytoplankton become very abundant late in the simulation because the predominant small cells are not efficiently grazed by either species of grazer.

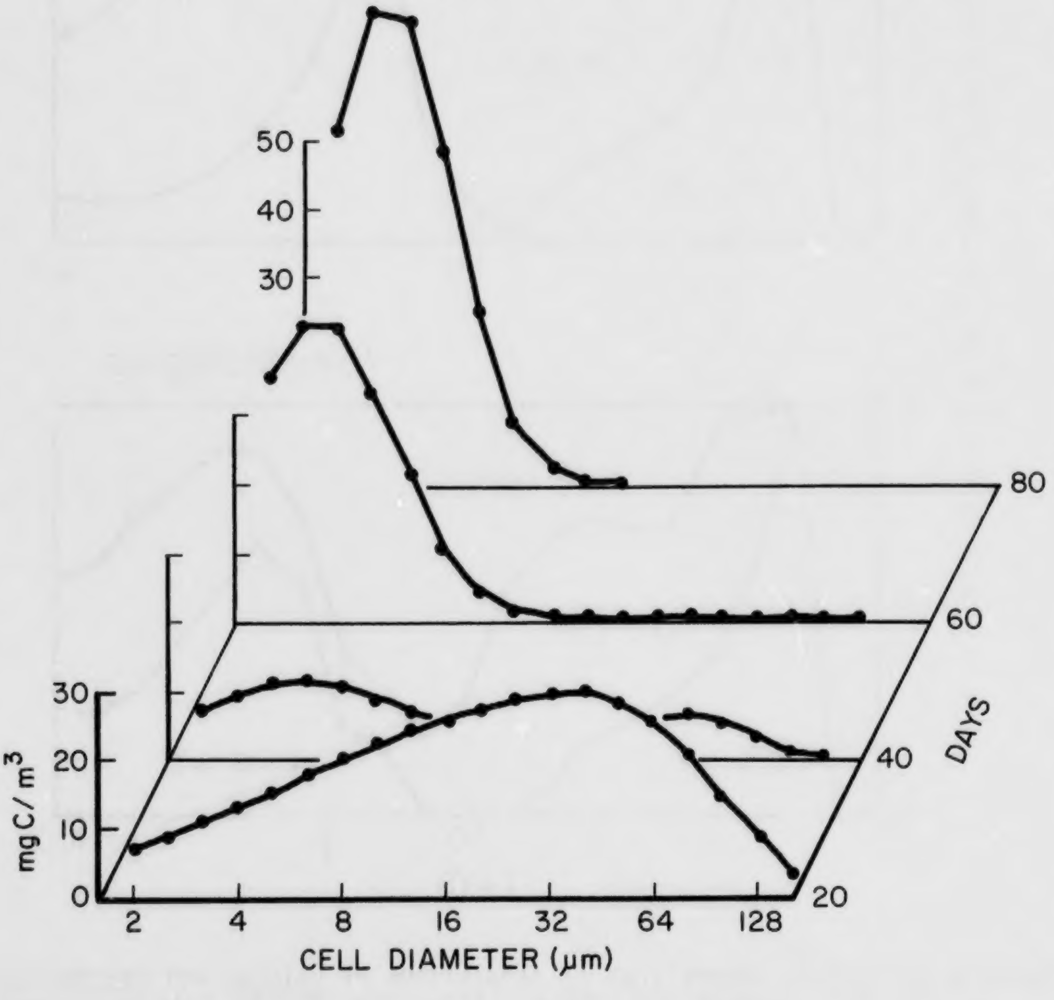


Figure 4. Size composition of the phytoplankton from day 20 to day 80 when the phytoplankton respiration coefficient a (eq. 1) is 0.24.

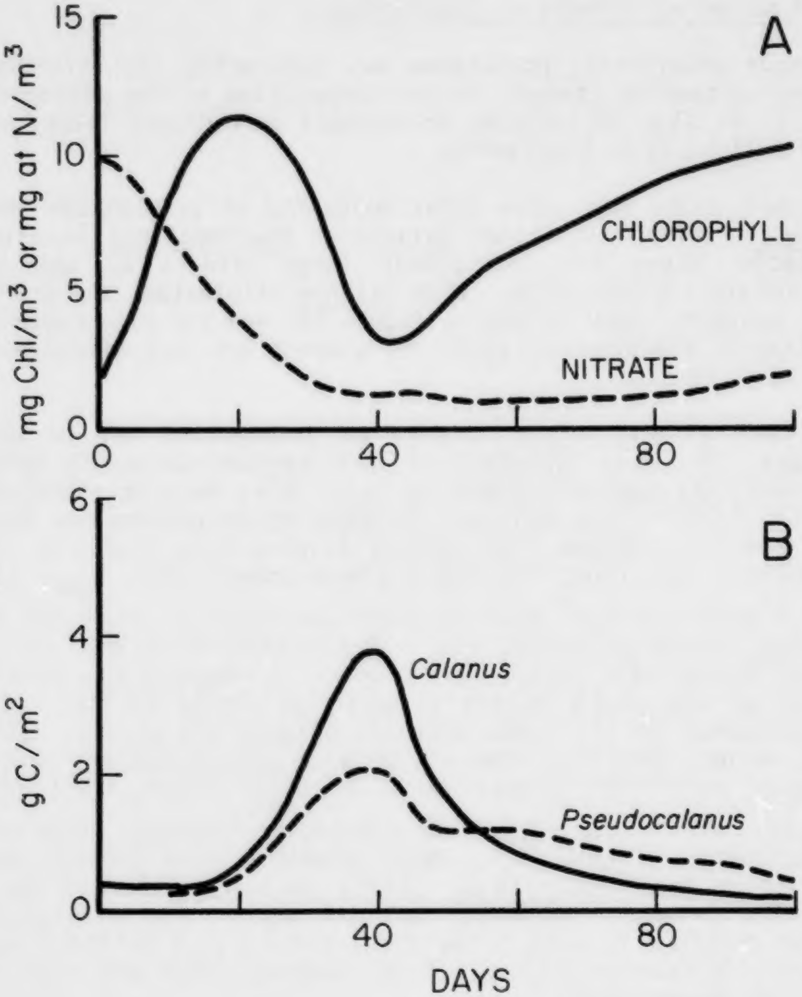


Figure 5. Changes in time of chlorophyll and nitrate (A) and zooplankton biomass (B) when the phytoplankton respiration coefficient is 0.24.

The shift in phytoplankton composition to small cells tends to favor the growth of the smaller species of grazer after the spring bloom (Figure 5B). In this simulation, Calanus does not reproduce after day 40 and, although a large population of copepods is produced in the first generation, they never recruit to the adult stage. On the other hand, first generation adults of Pseudocalanus recruit between days 35 and 40, and a second complete generation is produced before the end of the 100-day simulation.

Should a pollutant favor growth of some other type or size range of phytoplankton cells, the relative abundance of grazer species would change depending upon the abilities of the grazers to utilize the predominant phytoplankton size classes. In turn, the growth rates of predators of the grazers would also be affected (e.g., Koeller and Parsons 1977).

Lethal and Sublethal Effects on Zooplankton

As shown previously, pollutants may indirectly but strongly affect the zooplankton by causing changes in the composition of the phytoplankton. With the model it is also possible to investigate more direct lethal and sublethal effects of pollutants on zooplankton.

Some pollutants may cause total mortality of zooplankton species (e.g., Federle *et al*. 1979). If either *Calanus* or *Pseudocalanus* is eliminated from the *simulation* there are *concomitant* large effects on the dynamics and structure of the phytoplankton. With *Calanus* eliminated, the predicted phytoplank ton standing stock resembles *Figure 5A*, and the size composition of the phytoplankton is dominated by small cells which are less effeciently grazed by *Calanus* (Figure 6B).

Sublethal effects of pollutants on zooplankton may be manifested in several ways. Observed sublethal effects include decreased rates of growth and fecundity of copepods (Reeve *et al*. 1977; Moraitou-Apostolopoulou and Verriopoulos 1979). This may be *simulated* by decreasing the maximum growth rate of a species. Assume that *Calanus* is more sensitive than *Pseudocalanus* to a particular pollutant. If *the maximum growth rate*, G_{max}, *of Calanus* is decreased from 0.4 to 0.2, that is, the same value as for *Pseudocalanus*, then *Pseudocalanus* dominates during the phytoplankton bloom *but subsequently* the *composition of* the zooplankton shifts twice in response to change in the size composition of the phytoplankton (Figure 7). Early in the simulation (day 20), an abundance of all sizes of cells (Figure 7C) permits optimal feeding and growth of both species. However, because *Calanus* matures more slowly than *Pseudocalanus* with the assumed value of G_{max} *(Frost 1979)*, *Pseudocalanus* predominates. Near the middle of the simulation (day 60), large cells dominate the phytoplankton (Figure 7C), which promotes good growth conditions for *Calanus* but not for *Pseudocalanus*. Thus, the composition of the zooplankton *shifts* toward *Calanus*. *However*, near the end of the simulation (day 90), nitrate has *declined* to such a low level (<0.8 µg of N/liter) that growth of smaller cells is favored (Figure 7C) and feeding and growth conditions improve once again for *Pseudocalanus*.

Effects on Higher Trophic Levels

The final application of the simulation model is to examine some possible effects of pollutants which impact primarily the higher trophic levels such as the predators of the herbivorous zooplankton. The effects may be of two general types: An overall reduction in predation rate on both species, reflecting general decline in abundance of all predators, and a selective reduction in predation rate, reflecting qualitative shifts in the composition of the predator assemblage.

For the first case the simulation model was run with the predation coefficient m (eq. 2) decreased to 0.05, that is, half the value used in the standard simulation. Because of decreased predation, the zooplankton initially increase at a faster rate than in the standard simulation and the phytoplankton bloom is terminated sooner (Figure 8A,B; cf. Figure 1). Nitrate concentration

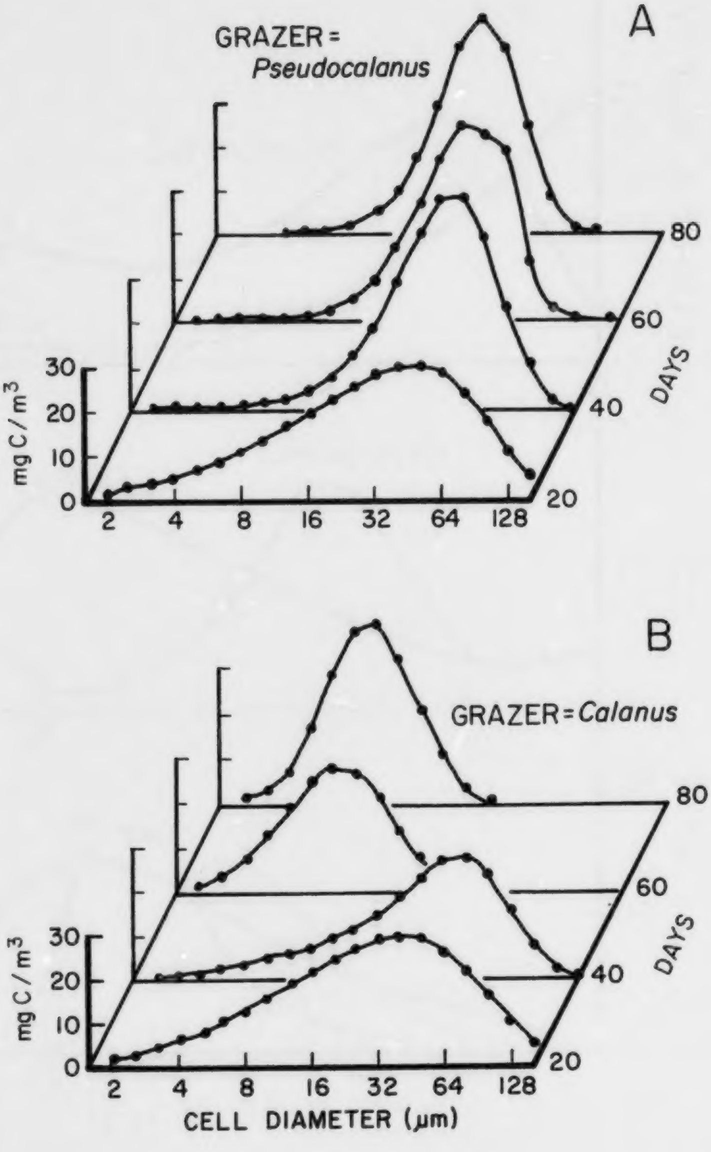


Figure 6. Size composition of the phytoplankton from day 20 to day 80 when Calanus is omitted from the standard simulation (A) and when Pseudocalanus is omitted from the standard simulation (B).

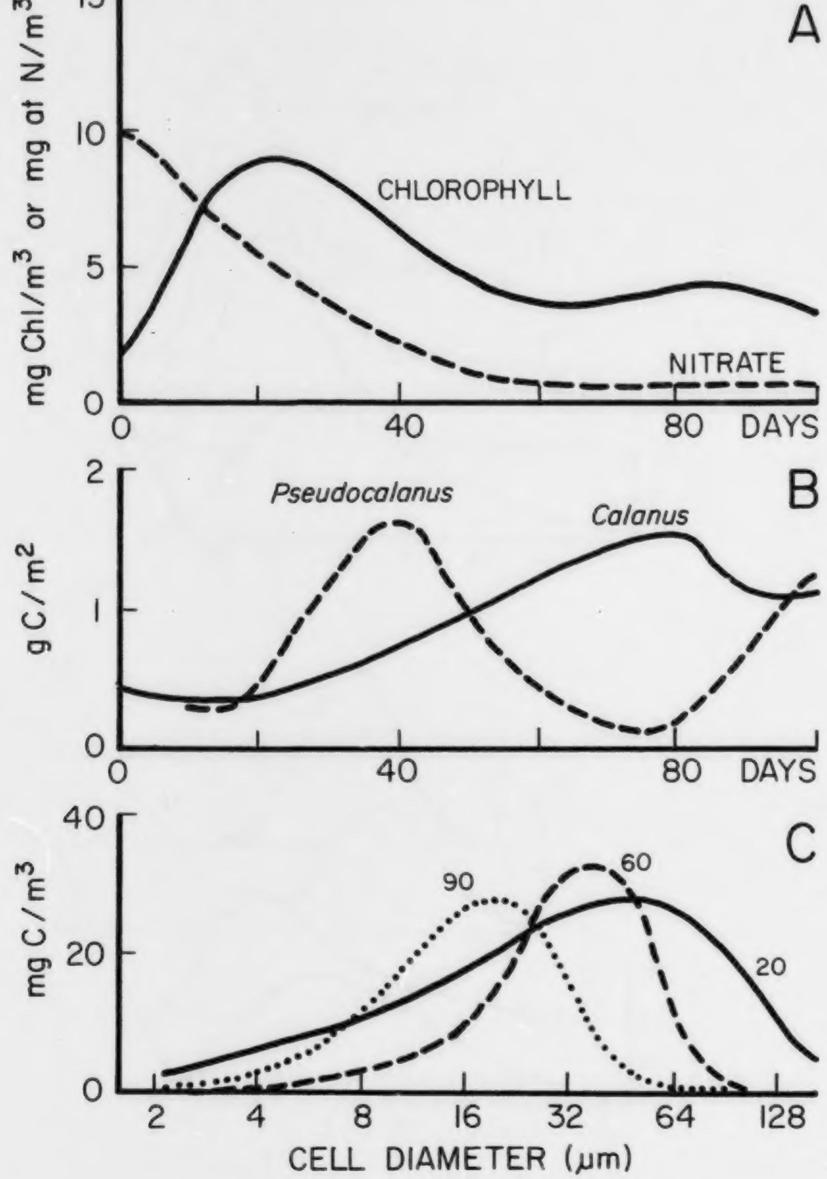


Figure 7. Simulated dynamics of plankton when the maximum specific growth rate, G_{max}, of Calanus is decreased to 0.2. A, changes in time of chlorophyll and nitrate. B, changes in time of zooplankton biomass. C, size composition of the phytoplankton at days 20, 60, and 90.

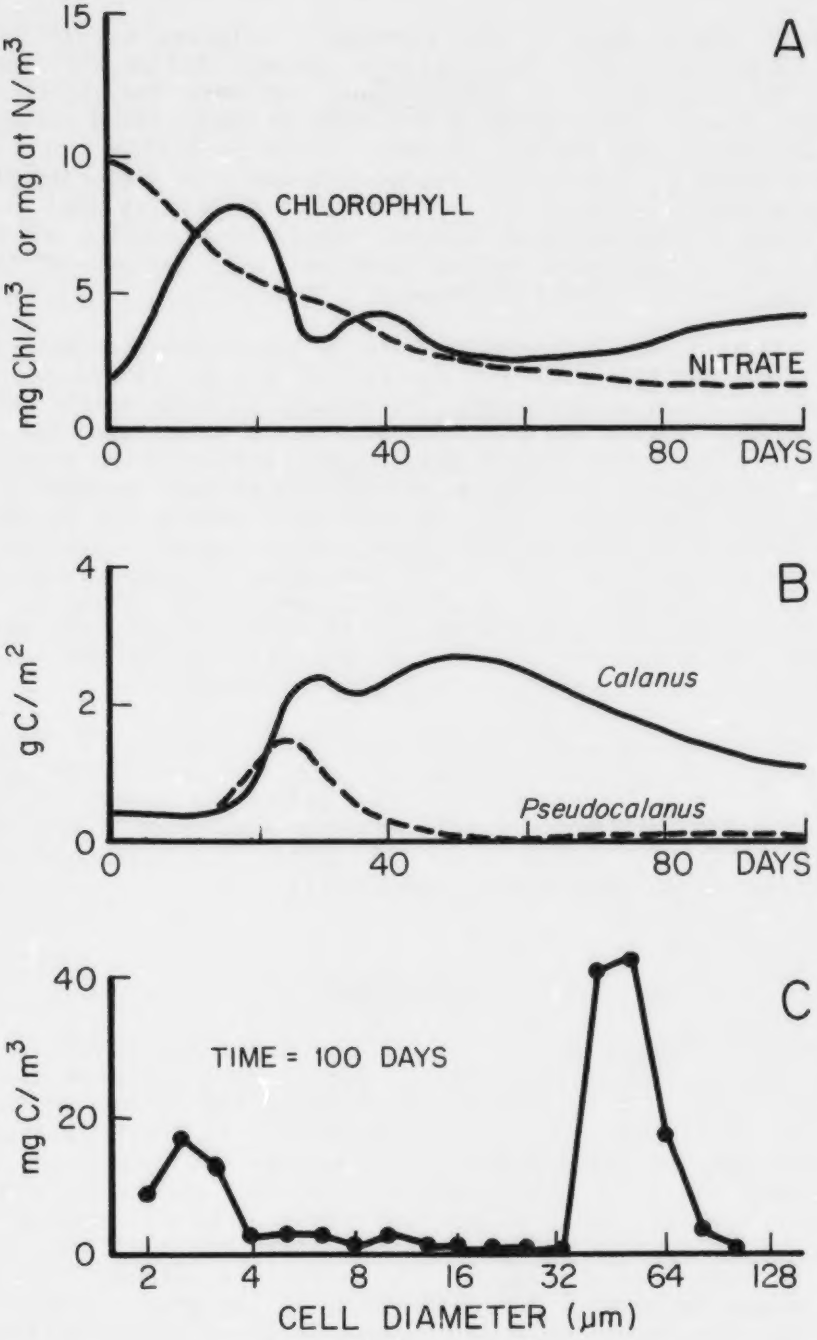


Figure 8. Simulated dynamics of plankton when the predation coefficient m (eq. 2) is decreased to 0.05. A, changes in time of chlorophyll and nitrate. B. changes in time of zooplankton biomass. C, size composition of the phytoplankton at day 100.

declines more slowly than in the standard simulation and this favors the growth of large cells which *Pseudocalanus* cannot utilize efficiently. Consequently, the population of *Pseudocalanus* declines drastically after the phytoplankton bloom. The increased abundance of zooplankton during the bloom seems to accelerate the process, evident in the late stages of the standard simulation (Figure 2), leading to strongly bimodal size frequency distribution of the phytoplankton (Figure 8C). The result of this is that in the later stages of the simulation both copepod populations consist of reproducing adults whose offspring starve in the naupliar stages because of the scarcity of phytoplankton cells in the size range of 4 to 32 μm.

Qualitative change in predation rate on the zooplankton was simulated by alternately varying the predation coefficient m (eg. 2) for one of the two grazer species. When m for *Calanus* is reduced to 0.05, while maintaining m for *Pseudocalanus* at 0.1, the predicted plankton dynamics are similar to those of the standard simulation except that *Calanus* dominates the zooplankton even more strongly throughout the simulation and is much more abundant than *Pseudocalanus* at 100 days (Figure 9A). By contrast, when m for *Pseudocalanus* is reduced to 0.05, it is favored over *Calanus* which, as in an earlier simulation (Figure 6A), results in a shift in the phytoplankton composition toward very large cells (Figure 9B). The predominant large cells cannot be efficiently grazed by the young developmental stages of either species and therefore the total zooplankton biomass gradually declines until at day 100 it is only 18% of that predicted for the standard simulation (Figure 9B).

In conclusion, quantitative and qualitative changes in predation rate strongly affect the growth dynamics of both the zooplankton prey and the phytoplankton. This reinforces the conclusions of Landry (1976), Steele (1976), and Steele and Frost (1977) that in simulation models of plankton dynamics the pattern of predation on zooplankton is as important as any other process in structuring the plankton assemblage.

DISCUSSION

The model of Steele and Frost (1977) provides one method for explicitly describing the population dynamics of species in a plankton assemblage. A model of this type will be required to simulate the effects of pollutants on the dynamics of plankton because species differ in their tolerance to pollutants. Yet even the present model is an extreme simplification. The assumption of Steele and Frost (1977) that size of cell determines growth rate of phytoplankton species, while in general correct, may misrepresent significant variations among species within a narrow range of size (see, for example, Figure 1 in Banse 1976). Indeed, the model has already been revised, as described above, to account for differences in the growth rate of species of copepods which are unpredictable from consideration of body size (Frost 1979).

The predictions of the model would also be very different if greater complexity were introduced into the herbivore and predator components. For example, inclusion of a microzooplankton component, grazing on the very smallest sizes of cells, would probably stabilize the structure of the zooplankton after the bloom. On the other hand, explicit description of population dynamics of a predator, such as a species of chaetognath or ctenophore, would very likely change the predictions in a material way, particularly if the predator were size--selective its feeding.

Finally, it is highly unlikely that pollutants would affect only the members of one trophic type. In reality, one could expect stress of several modeling of effects of pollutants will therefore require extensive knowledge on different trophic types.

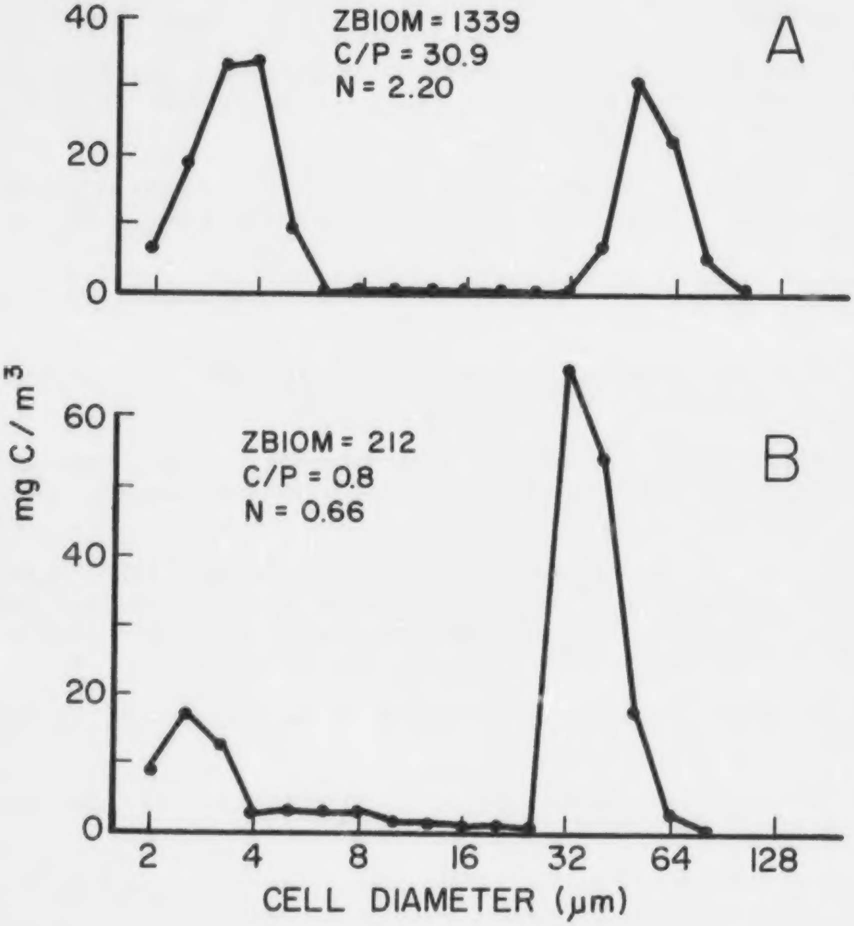


Figure 9. Size composition of the phytoplankton from day 20 to day 80 when Calanus is omitted from the standard simulation (A) and when Pseudocalanus is omitted from the standard simulation (B).

REFERENCES

Banse, K. 1976. Rates of growth, respiration and photosynthesis of unicellular algae as related to cell size - a review. J. Phycol. 12:134-140.

Evans, G. T., J. H. Steele, and G. E. B. Kullenberg. 1977. A preliminary model of shear diffusion and plankton populations. Scottish Fish. Res. Rept. No. 9.

Federle, T. W., J. R. Vestal, G. R. Hater, and M. C. Miller. 1979. Effects of Prudhoe Bay crude oil on primary production and zooplankton in Artic tundra thaw ponds. Mar. Environ. Res. 2:3-18.

Frost, B. W. 1979. The inadequacy of body size as an indication of niches in the zooplankton. In The Evolution and Ecology of Zooplankton Populations, W. C. Kerfoot (ed.). Amer. Soc. Limnol. Oceanogr. Spec. Sympos. III.

Greve, W., and T. R. Parsons. 1977. Photosynthesis and fish production: Hypothetical effects of climatic change and pollution. Helgolander wiss. Meeresunters 30:666-672.

Koeller, P., and T. R. Parsons. 1977. The growth of young salmonids (Oncorhynchus keta): Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:114-118.

Landry, M. R. 1976. The structure of marine ecosystems: An alternative. Mar. Biol. 35:1-7.

Menzel, D. W. 1977. Summary of experimental results: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:142-145.

Moraitou-Apostolopoulou, M., and G. Verriopoulos. 1979. Some effects of sub-lethal concentrations of copper on a marine copepod. Mar. Pollut. Bull. 10:88-92.

Reeve, M. R., M. A. Walter, K. Darcy, and T. Ikeda. 1977. Evolution of potential indicators of sub-lethal toxic stress on marine zooplankton (feeding, fecundity, respiration, and excretion): Controlled excosystem pollution experiment. Bull. Mar. Sci. 27:105-113.

Steele, J. H. 1976. The role of predation in ecosystems models. Mar. Biol. 35:9-11.

Steele, J. H., and B. W. Frost. 1977. The structure of plankton communities. Phil. Trans. R. Soc. Lond. B 280:485-534.

Stockner, J. G., and N. J. Antia. 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients, and pollutants -- a warning. J. Fish. Res. Bd. Canada 33:2089-2096.

A QUANTITATIVE METHOD FOR EVALUATING EXTERNAL EFFECTS ON ECOSYSTEMS

by

V. D. Fedorov, V. N. Maksimov, and V. B. Sakharov
Moscow State University

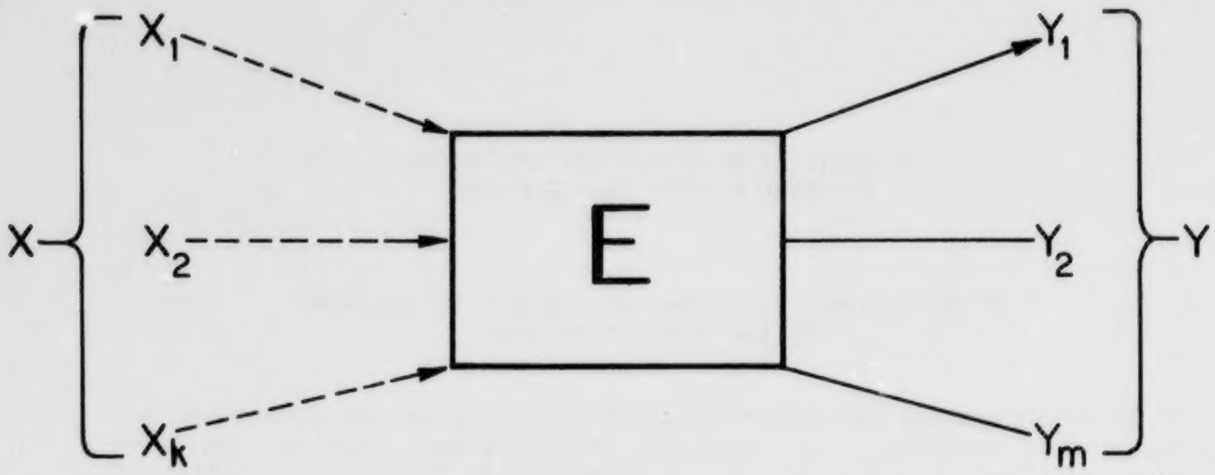
Different methods for evaluating external effects on the environment, and ecosystems in particular, are discussed in this paper. However, there are various difficulties which can only be surmounted by significant intellectual and material efforts.

Questions concerning methods for evaluating the state of ecosystems, specifically regarding their productivity, their ability to resist harmful impacts, the preservation of their characteristics, and the potential for exploitation are still debated. The situation is aggravated by the need to overcome these problems soon if the balance between man and nature is to be preserved. The complexity of the situation has fostered different approaches, each of which has advantages and disadvantages. We shall discuss one method for evaluating the state of ecosystems that was recently developed in the Department of General Ecology and Hydrobiology at Moscow State University (USSR).

STATEMENT OF THE PROBLEM

We shall examine a model of an ecosystem to test for the effect of pollutants (see diagram). The center square represents an ecosystem, formed by a set of populations which interact with each other and the biotope, each of which is sufficiently heterogeneous in internal structure (sex, age, function) and performs a similar or dissimilar function in biocenosis.

Transformation of energy and metabolism occurs within this ecosystem, i.e., it is sufficiently autonomous and complex and has, like any spatially defined entity, a series of integral characteristics which determine its composition (set of populations), structure (interaction of population) and changes in function (population shifts, changes in their relationships). Indices of the resistance (conditioned by homeostasis), stability (conditioned by maturity) and complexity (conditioned by the species difference and the difference in the relationships between populations) are included in these integral characteristics. The potentially harmful substances are indicated by the broken arrows on the left, as the "input" into the system. It is hypothesized that there is a small number for each real ecosystem, that the number can be determined approximately, and that the physical-chemical features, i.e., the set of pollutants, will differ and vary from ecosystem to ecosystem.



Diagram

It is known a priori that some portion of pollutants will "interact" with each other in their effect on the ecosystem. This means that the effect of each one individually will be substantially attenuated or potentiated as a function of the effect of any other pollutant or pollutants.

The so-called indices of the state of the ecosystem, both with respect to the biotic and abiotic parts, are indicated by the solid arrows to the right, at the "output" of the ecosystem. Since the number, based on the condition $k \gg m$ [translator's note: handwritten notation illegible], we will eliminate a limited number of indices which describe the ecosystem as "good" or "poor" as a result of the effect of k pollutants based on some rules (it is assumed that they have a satisfactory basis). We can then consider such indices of the type as quality indices if the quality is determined by a set of properties of the ecosystem which we consider desirable.

The diagram thus generally reflects the multiple effects of the pollutants on the ecosystem whose condition is determined by multiple responses selected as a function of human interests (e.g., indices of the quality of drinking water) or in the interests of the ecosystem itself (e.g., preservation of the productivity characteristic of the given type of ecosystem). In this case, the problem is formally reduced to studying the following type of function:

$$\bar{y} = f(\bar{x}) \tag{1}$$

In developing a strategy for biological monitoring, we previously suggested specific procedures for establishing priority among the set of interacting factors and determined the need to conduct a multifactor experiment as a basic method for obtaining information on predicted consequences of pollutants on the ecosystem (Fedorov 1976, 1977).

Matrices of first and second order plans (Maksimov and Fedorov 1969; Golikova et al. 1974) were proposed as an experimental design which would allow independent, simultaneous investigations of a combination of n selected variables. These schemes are now routinely used in the form of a polynomial. Thus, we can consider these methods and approaches for studying a set of independent variables as well developed and tested.

WHAT ARE THE DEPENDENT VARIABLES AND HOW SHOULD THEY BE INVESTIGATED?

An ecosystem has many features which determine its composition, structure, function, development, maturity, resistance, etc. Within the framework of our task, these can be considered dependent variables. Since the number of dependent variables significantly exceeds the number of active elements ($k >> m$), the need to limit the number of indices describing the condition of the ecosystem becomes apparent.

Evaluations related to processes (related to the differences in types and the force of interaction between organisms) and the results of these processes (expressed by the relationships of the nature or biomass of the populations forming the "face" of the biocenosis) are the basic indices of the structure of ecosystems.

In addition, the need to add an indicative approach to the series of new principles for describing the structural features of natural ecosystems seems obvious. In this respect, regardless of whether the different indices are related to estimates of the rate of the processes or to their results, we shall examine the recent attempts to improve the estimates of pollution of natural waters.

The first group of estimates consists of indices which could be expressed by an integral in time, i.e., as some result of the effect (functions) at the time they were recorded. This category includes the indices which describe the size of the biomass, the number of species, the relationships of the members, etc., and also the different indices for species resources, variety and equivalence (expression), the relative abundance, domination, etc., calculated on this basis.

One of the most popular and successful models for evaluating water pollution based on a series of indices concerning the benthic fauna is the Woodwiss method developed for the Trent River (Woodwiss 1964). Having significantly reduced the list of indicative organisms, Woodwiss introduced the concept of "group"--usually for denoting easily determined forms: in some cases, species; in others even families. The principle of the indicative value of such taxa is supplemented by the principle of a decreased variety of fauna in polluted water.

The second group of evaluations of the structure of ecosystems is composed of indices which can be expressed by a derivative in time, i.e., as the rate of change in some function. This category includes indices of productivity, respiration, assimilation of substances, and other features of the processes which take place in the ecosystem. The number of these indices is large, thus, in order to monitor or judge the condition of the ecosystem, it is necessary to select a few representative indices using any discriminatory criterion. Earlier (Federov 1977), we formulated the basic requirements for determining dependent variables and listed some reference indices describing the condition of an ecosystem. We examined the possible situation where certain combinations of "normal" and "pathologic" individual indices are specific with respect to different pollutants and consequently the characteristics of the combinations can be considered symptoms of a specific disease in the ecosystem.

It is possible to examine the opposite situation which includes the absence of any specific effect of the various pollutants, at least for indices which describe the flow of energy and substances in the ecosystem. In this case, the investigation of the principles of behavior of the individual features permits interpretations of whether they are "good" or "bad" for the function and existence of the ecosystem. The analysis of curves or a set of data relating to evaluations of the intensity of a process essentially only provides information concerning the quality of the index which we consider demonstrative in judging whether this is "good" or "bad" for the system. The need then arises to combine indices, some of which indicate the good quality of the envionment or condition of the ecosystem and others which indicate the poor condition of the ecosystem. Two successive stages are implied in solving the problem which arises in this way: The first requires introducing an evaluation of the quality of each dependent variable d_i; the second involves evaluating the quality D based on the results of evaluations of selected indices d_i.

The features associated with d_i and y_i can be established by "common sense" which absolutely rejects the presence of a linear correlation between them, since the region of a sharp change in desirability in the norm implies the existence of a region of more serious changes in function.

Harrington (in Maksimov 1977) has introduced the nonlinearity of the formula on a continuous scale:

$$d = e^{-e^{-z_i}} \qquad (2)$$

where z_i is a coded variable which can easily combine a linear function with any real variables y_i. The general form of function 2, which resembles a logistics curve, is shown in Figure 1.* It is necessary to consider the fact that the value $z = 5$; $d = 0.98$ with $z = 4$; $d = 0.5$ with $z = 1.5$; $d = 0.68$ with $z = i$; $d = 0.37$ with $z = 0$; and finally, $d = 0.0006$ with $z = -2$ correspond to desirability of 0.993 (i.e., very close to one). For discovering the correlation of z with real dependent variables y_i, it is sufficient to combine the limits of the normal variability of the dependent variables with the range of changes in z from -2.2 to 5.

---

* (Editor's note: figure not reproducible).

The detailed basis of the approaches used by Harrington's school to evaluate the quality of biologic systems is given by Maksimov (1977). However, Harrington's function is not adequate to evaluate the condition of natural systems due to the monotonic nature of the correlation between d_i and y_i which it describes. In reality, the type of function should be unimodular in biology in general and in ecology in particular, i.e., the curve for the function should have one maximum corresponding to the optimum desirability. If we use "more-frequently-better" as the initial position (instead of "greater-better"), the desirability of any index can be determined by knowing its distribution function.

Theoretically, distribution functions should in some way be related to the concept of a statistical standard, but this question has not been investigated since the concept of statistical standard is based on a hypothesis and is only intuitively perceived (Fedorov 1977). Regardless of the nature of the distribution, desirability equal to "1" can always be designated to a model class value and, in this case, the basic difficulty is reduced to making a decision concerning the rule for selecting the range to the left or right of "1."

If we know the limits of the normal variability of the variable, it is possible to combine each index for the desirability value equal to 0.63 with any "extreme" normal value which corresponds to the lower repartition of the concept "good" on Harrington's scale.

Many different methods can be used for determining the "extreme" value; two are shown below.

The first method is borrowed from medical practice and is based on the percentile method (Sepetliyev 1968). Any response values within the limits of the 25th to 75th percentile should be considered normal, and a comparison of the actual values of these percentiles for each response with a given desirability value will provide the necessary bond between any real index and its desirability value.

The second method is based on knowledge of the law of the distribution of response indices in an intact system and the assumption that we can actually determine the evaluation of the mean ("a") and the evaluation of the deviation from the mean (σ). The assumption of a hypothesis of normality (if necessary, "normalization" should be obtained by taking the logarithm of the results) allows isolating the region of "normal" values located within the limits of "a" - σ to "a" + σ, corresponding to the evaluation of "good" and including approximately two-thirds of the response evaluation figures.* The region of the values for variables assigned by condition "a" ± 2σ will also include "bad" evaluations, indicating an alarming state of affairs in the ecosystem.

---

* Editor's note: figure not reproducible.

In order to judge the "poor" or "good" state of the ecosystem as a whole, we should use a generalized desirability index which could be calculated as the mean geometric set of evaluations d_i

$$D = \sqrt[n]{d_1, d_2 \ldots d_m} \qquad (3)$$

where d_i are the coded values of the particular desirability values found by one of the methods described above.

TABLE 1. RECOMMENDED INTERVALS IN THE DESIRABILITY SCALE IN A 5-POINT SYSTEM FOR EVALUATING THE WELL-BEING OF ECOSYSTEMS

D	State	Points
1.0 - 0.8	Excellent	5
0.8 - 0.6	Good	4
0.6 - 0.4	Fair	3
0.4 - 0.2	Poor	2
0.2 - 0.0	Very Poor	1

AN EXAMPLE OF EVALUATING THE STATE OF ECOSYSTEMS USING THE DESIRABILITY FUNCTION

Calculation of the desirability values is based on the hydrochemical data given in the Woodwiss article (1977). In this study, the results for determining the biotic index in different sections of the Trent River and a number of its tributaries are compared with the values of the most important hydrochemical indices measured at the same stations.

Based on the graphs in the Woodwiss work, graphs were plotted to convert the natural values of the selected indices to their desirability values.

The generalized desirabilities were calculated for 49 stations based on the data cited in Woodwiss' work. The individual desirability values for all three indices and the generalized desirability values D calculated on their basis are shown in Table 2. This table also shows the biotic index value determined by Woodwiss at these stations for comparison. Based on his statement, the deviations in estimating the index usually do not exceed 1 point on the scale. Within these limits, the desirability value D, multiplied by 10, coincides with Woodwiss' index in 34 cases, and the difference between the desirability and the index exceeds 2 units in only four cases. This agreement of the data should be considered totally satisfactory.

In the example, the desirability function was used to evaluate the effect of different pollutants on an ecosystem. The results of an experiment whose purpose was to determine the effect of two petroleum products--diesel fuel and motor oil--and a dispersion substance--correxite 7664--on White Sea plankton

TABLE 2

Station No.	Total Permanganate Oxidizability (/mg/liter) and and Its Desirability		Ammonium Nitrogen (/mg/liter) and Its Desirability		Dissolved Oxygen and Its Desirability (/mg/liter)		Desir-ability	Biotic Index	Difference
1	2	3	4	5	6	7	8	9	10
1	5.2	0.68	0.4	0.34	9.9	0.88	0.80	7	1.0
2	6.7	0.53	3.7	0.29	7.0	0.37	0.33	4	0.2
3	7.9	0.43	2.1	0.46	8.1	0.46	0.45	5	0.5
4	6.2	0.58	0.4	0.84	8.1	0.46	0.61	6	0.1
5	7.3	0.48	2.4	0.42	6.3	0.32	0.40	5	1.0
6	6.2	0.58	0.6	0.78	9.2	0.64	0.66	6	0.6
7	4.6	0.74	0.2	0.92	10.3	0.92	0.86	8	0.6
8	8.8	0.36	9.1	0.09	6.0	0.30	0.21	2	0.1
9	8.9	0.36	1.2	0.62	7.2	0.39	0.44	6	1.6
10	2.0	0.93	0.0	1.00	11.7	1.00	0.98	10	0.2
11	3.8	0.81	1.5	0.56	6.3	0.32	0.53	6	0.7
12	3.2	0.85	0.1	0.96	12.5	1.00	0.93	8	1.3
13	1.5	0.96	0.1	0.96	12.4	1.00	0.98	10	0.2
14	7.5	0.46	1.5	0.56	9.5	0.74	0.58	4	1.8
15	8.0	0.42	5.4	0.17	6.4	0.32	0.28	2	0.8
16	6.3	0.57	4.3	0.24	10.2	0.92	0.50	7	2.0
17	4.8	0.72	0.4	0.84	11.1	0.99	0.84	8	0.4
18	6.4	0.56	7.4	0.11	9.8	0.86	0.38	4	0.2
19	2.8	0.83	0.0	1.00	13.7	1.00	0.96	7	2.6
20	6.0	0.60	1.2	0.62	9.3	0.68	0.63	6	0.3
21	4.8	0.72	0.5	0.83	9.4	0.74	0.76	8	0.4
22	3.6	0.38	3.8	0.28	5.6	0.28	0.31	3	0.1
23	4.6	0.74	0.2	0.92	9.8	0.86	0.84	6	2.4
24	6.0	0.60	1.9	0.49	7.7	0.43	0.50	3	2.0
25	5.0	0.70	1.5	0.50	7.4	0.40	0.54	5	0.4
26	5.0	0.70	1.4	0.58	7.1	0.37	0.53	4	1.3
27	6.0	0.60	2.3	0.37	6.3	0.32	0.41	4	0.1
28	4.0	0.79	0.2	0.93	10.9	0.98	0.90	10	1.0
29	3.0	0.87	0.2	0.93	10.8	0.97	0.92	10	0.8
30	4.0	0.79	0.2	0.93	9.6	0.79	0.88	10	1.7
31	4.0	0.79	0.3	0.83	10.7	0.96	0.87	9	0.3
32	3.0	0.87	0.3	0.83	18.4	1.00	0.91	9	0.1
33	5.0	0.70	0.3	0.88	9.2	0.64	0.73	9	1.7
34	5.0	0.79	0.8	0.72	9.5	0.78	0.73	7	0.3
35	4.0	0.70	0.8	0.72	9.1	0.62	0.71	7	0.1
36	5.0	0.79	1.6	0.55	8.3	0.48	0.57	6	0.3
37	5.0	0.60	0.9	0.69	8.4	0.49	0.62	4	2.2
38	6.0	0.60	2.1	0.47	6.0	0.30	0.44	4	0.4
39	6.0	0.50	1.2	0.62	8.1	0.46	0.56	4	1.6
40	7.0	0.35	3.0	0.67	7.8	0.44	0.53	2	3.3
41	9.0	0.79	2.7	0.39	6.7	0.34	0.36	2	1.6
42	4.0	0.60	0.3	0.88	10.5	0.94	0.87	8	0.7
43	6.0	0.70	0.4	0.85	9.6	0.73	0.74	8	0.6
44	5.0	0.29	0.5	0.82	10.6	0.93	0.82	8	0.2
45	10.0	0.29	6.2	0.14	6.3	0.32	0.24	4	1.6
46	10.0	0.29	7.7	0.10	6.1	0.30	0.21	2	0.1
47	10.0	0.29	6.2	0.14	7.6	0.41	0.26	3	0.4
48	10.0	0.29	5.3	0.18	7.0	0.37	0.27	3	0.3
49	12.0	0.20	7.1	0.12	6.6	0.84	0.20	3	0.1

when certain combinations of the substances were added according to a plan for total factor experiment (TFE) 2^3 were used. The state of the plankton was evaluated based on changes in the number of the five species of algae and four types of zooplankton which dominated at the time the experiments were conducted (July 1975) and based on changes in production indices in fractions of the controls, set at 1 for all 11 response functions (Table 3).

TABLE 3

	1 Controls	2	3	4	5	6	7	8
Concentration, mg/l								
Diesel fuel x_4	0	10	0	10	0	10	0	10
Motor oil x_2	0	0	10	10	0	0	10	10
Correxite x_3	0	0	0	0	10	10	10	10
Ratio of Experimental Values to Control Values								
Scelentonema costatum y_1	1.00	0.10	0.09	0.08	0.98	0.17	0.07	0.07
Small flagellata y_2	1.00	0.06	0.12	0.04	0.92	0.06	0.63	0.05
Chaetoceros wighamil y_3	1.00	0.12	0.28	0.16	0.89	0.21	0.29	0.21
Cyanobacteria y_4	1.00	0.16	0.60	0.12	0.12	0.97	0.10	0.16
Dynabrion pellucidum y_5	1.00	0.10	0.06	0.11	0.19	0.10	1.00	0.14
Microzetella norvegica y_6	1.00	0.95	0.97	0.86	0.98	0.88	0.93	0.75
Oitona similis y_7	1.00	0.92	0.57	0.32	0.79	0.50	0.19	0.10
Acartia longiremis y_8	1.00	0.14	0.99	0.14	1.00	0.14	0.99	0.12
Temora longicornis y_9	1.00	0.48	0.98	0.41	0.99	0.48	0.98	0.41
Primary production y_{10}	1.00	0.82	0.82	0.56	0.93	0.32	0.94	0.10
Tempo assimilation CO_2 y_{11}	1.00	0.25	0.70	0.93	0.92	0.95	0.72	0.34
Overall desirability D	1.00	0.24	0.39	0.22	0.81	0.24	0.49	0.15
Factor symbols	"1"	x_1	x_2	x_1x_2	x_3	x_1x_3	x_2x_3	$x_1x_2x_3$
Regression coefficients b_i	0.442	-0.230	-0.130	0.102	-0.020	0.002	0.028	-0.045

The relationship between each of these functions and the three factors studied generally does not coincide with the rest. If the number of diatoms decreases under the effect of the petroleum products, the growth of blue-green algae will be significantly stimulated with certain values of impurities, with which the increase in primary productivity in those variants of the experiments where diesel fuel was added is apparently related. The different types of zooplankton significantly differed with respect to sensitivity to the petroleum products and the correxite.

Desirability functions can be used to conduct a complete evaluation of the effect of the pollutants on the entire set of indices characterizing the condition of the plankton. In this respect, a basic difficulty exists in selecting the scale for converting the actual response function values to their desirability values. It is more or less clear that it would be more desirable to consider a situation where there is generally no harm in evaluating the damaging effect of external factors on the system. This means that the value of the response function in the controls should be used for 1 on the desirability scale. Establishing such a value for the response function in which the desirability should be considered equal to 0 is less specific. In particular, for a number of individual species in a community, decreasing them to 0, i.e., the total death of the population, is naturally an extremely undesirable phenomenon, but it is also clear that decreasing the number of species to some critical value, beyond which the population cannot recover even after the harmful effect has been eliminated, is no less undesirable. Since the critical values for the number of real species in real ecosystems is still unknown, we selected some intermediate point whose position can be determined on the basis of any biologically plausible hypotheses for constructing the desirability scales. In our example, we used the rather widely held opinion that a two-fold decrease in the number of the population with one harmful effect should be considered acceptable in the sense that this population preserves the capacity to return to the initial state after the pollution has been eliminated (e.g., due to self-purification of a reservoir). We extended this hypothesis to the productive indices due to the absence of any other sufficiently plausible hypotheses. Indices which were higher in the experiments than the controls, i.e., indices for which $y_i > 1$ were doubled in comparison with the controls.

In this context, "acceptable" means a deviation from the control which corresponds to an evaluation between "good" and "excellent" on the desirability scale. In our case, we selected the numerical value of the desirability $d = 0.85$ for quantitatively expressing this evaluation. The value of the arbitrary variable $z = 1.9$ corresponds to this value. We used $z = 5.0$ for the values of y_i in the controls; this gives a value of $D \approx 1$ in using formula (2). In selecting these "reference points," the values of y_i can be converted to z values by using the linear transform

$$z = \frac{P - 0.2}{0.16} \text{ , where}$$

$p = u$ when $y \leqq 1$.
$p = 1/y$ when $y > 1$.

Table 2 shows the desirability values calculated by this method.

The same table shows the values of the regression coefficients calculated according to the values for the three factors studied and their interactions [verification of the significance using Daniel's method [Daniel 1959)] showed that diesel oil had a more pronounced effect than motor oil on plankton (based on the total of 11 selected indices) in certain hypotheses concerning the desirability of changes caused by additives. The effect of each of them was greater separately due to the presence of a positive effect of their interaction. This interaction should be considered the sign of a monotypic nonlinear "dose-effect" function for the two substances; as a result, adding each of them to a system which is already subject to the inhibiting effect of the second does not cause an equally great effect. A similar case has been previously examined (Maksimov 1977).

The effect of correxite on the value of the desirability function, like the effect of its interaction with the petroleum products, was insignificant; it is thus possible to conclude that a 10 mg/liter concentration of correxite does not cause any significant undesirable changes in plankton and does not alter the toxicity of the petroleum products if "toxicity" is defined as the ability of a substance to cause undesirable changes in a community or ecosystem as a whole.

CONCLUSION

Using the existing methods for evaluating standards, and establishing the extreme limits of "good," etc., it is doubtful whether it is expedient to attempt to improve a proposed system for evaluating the state of an ecosystem. It can be accepted or rejected on the basis (again!) of a totally subjective opinion concerning the fact that it is worse or better than other evaluation systems. Attempting to combine the different schemes or, on the other hand, allowing them to exist in parallel and independently if knowing the logical basis and structure of the design is the only criterion for their existence, could also be justified.

It is doubtful whether any one system could be developed which would be better than all of the others and which would consequently be preferred in analyzing the conditions of ecosystems. As a function of human goals, the features of the conditions and communities and the methods of evaluation will be different in each case. However, the principles on which the methods of evaluation are based cannot be numerous. Based on general considerations, it would seem that all methods will concern the features of the functional-spatial structure of ecosystems or their living components to a greater or lesser degree. The integrity of such structures as ecosystems cannot be based on the features of their organization. The methods of evaluation will be easier to vary as a function of the type of ecosystem, its maturity, and "resistance reserves" (resistance) when they are used by humans. With respect to toxicologic evaluations of the quality of water, despite their primitivism and low price, their role in making ecologically based decisions cannot be decisive.

SUMMARY

The problem of evaluation of the state of the ecosystem on the basis of many structural and functional indices has been considered. Rules for choosing these indices during the elaboration of the system of control of the quality of the environment are suggested. The main difficulty in carrying out such a control was shown, namely: the numerous response functions and inde-

pendent variables. Establishing a generalized criterion for the state of the ecosystem which unifies individual indices makes it possible to overcome this difficulty.

The use of the so-called desirability function is proposed as one approach to the solution of this problem. The principles of the transition of the concrete indices to the conditional scales of desirability are also described in the paper. The practical application of the proposed approach is shown by two specific examples.

REFERENCES

Fedorov, V. D. 1976. Problems in the maximum admissible effects of the anthropogenic factor with respect to the ecologist. In Comprehensive Analysis of the Natural Environment. Gidrometeoizdat Press, Leningrad.

Fedorov, V. D. 1977. The problem of evaluating the normal and pathologic condition of ecosystems. In Scientific Principles for Controlling the Quality of Surface Waters Based on Hydrobiologic Indices. Proc. Sov.-Eng. Seminar, Gidrometeoizdat Press, Leningrad.

Golikova, T. I., L. A. Panchenko, and M. Z. Fridman. 1974. List of second order plants. Izd-vo MGU.

Maksimov, V. N., and V. D. Fedorov. 1969. Mathematical planning of biologic experiments. In Mathematical Methods in Biology 1968. Izd-vo VINITI, Moscow.

Maksimov, V. N. 1977. Specific problems in studying the combined effect of pollutants on biologic systems. Gidrobiol. Zhurn. 13(4).

Sepetliyev, D. A. 1968. Statistical methods in scientific medical studies. Meditsina Press, Moscow.

PETROLEUM POLLUTION AND MICROFLORA IN MARINE ECOSYSTEMS

by

M. V. Gusev, T. V. Koronelli and V. V. Il'inskiy
Department of Biology
Moscow State University

INTRODUCTION

Petroleum and petroleum products have become integral components of the marine environment. In undergoing microbiologic oxidation, these compounds enter the natural cycle and their presence has a definite effect on the microflora of ecosystems.

Since petroleum products are almost insoluble in water and are less dense than water, their distribution in the ocean is not uniform. There are critically polluted areas (petroleum discharges resulting from accidents, ports, petroleum output regions, outfalls discharging polluted water) where the surface of the ocean is completely or partially covered by a film of petroleum; moderately polluted areas where petroleum cannot be visually detected and there is no film, but the concentration of petroleum products exceeds the maximum admissible quantity (open waters of the ocean); and finally, there are sections which are free of petroleum and the concentration of petroleum products is below the maximum admissible concentration (some of the world unperturbed by man). The effect of petroleum pollution on microflora is a function of its intensity. For this reason, our studies involved areas of the ocean with different degrees of pollution: ports and polluted coastal waters, the open sea and regions totally free of pollution.

The following aspects of the problem were emphasized:

(1) Determining the quantitative mechanisms of the distribution of petroleum-oxidizing microorganisms and microorganisms from other groups;

(2) Evaluating the possibility of indicating petroleum pollution using specific microflora;

(3) Determining and studying the active petroleum-oxidizing microorganisms.

The regions investigated were generally located in the northern latitudes. They included the Finnish Straits of the Baltic Sea, the Arctic Ocean of the USSR (White, Barents, Kara, Laptev Seas), the coastal waters of Wrangel Island, the coastal waters of the Komandorskiy Islands, the northwestern part of the Pacific Ocean (Figure 1).

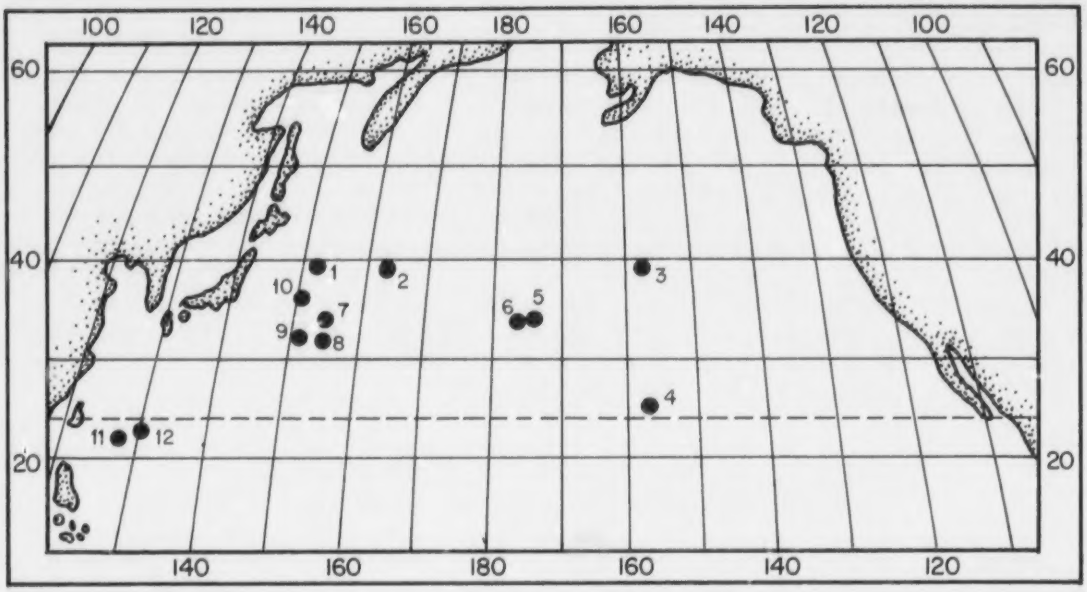


Figure 1. Location of the sampling sites in the Pacific Ocean.

We note that similar studies have been conducted previously in the regions of the Soviet Arctic. However, pollution from petroleum products represents the greatest hazard in these latitudes, since petroleum biodegradation processes are slow at low temperatures. The quantitative mechanisms in the distribution of petroleum-oxidizing microflora and other microorganisms were studied in the Arctic Seas, the coastal waters of the Komandorskiy Islands, and the northwestern section of the Pacific Ocean. Water samples from all of these regions, and samples from the coastal waters in the Finnish Straits of the Baltic Sea and the region of Wrangel Island were used to determine and investigate the active petroleum-oxidizing microorganisms.

METHODS

In studying the coastal waters, the samples were taken from the surface layer with a 100-ml sterile bottle; in open waters the samples were taken with a Zobell bathometer.

In working in the open waters of the Pacific Ocean (October-December 1977), the samples of water were taken from a boat which was 1 km away from the ship. Samples of the surface film were taken with a sterile capron screen, and samples were taken from the meter level with Zobell bathometer.

The water temperatures during the investigation period were as follows: 0 to 6° in the Arctic Seas (surface partially covered with ice), 2 to 4° in the coastal waters of the Mednyy I. (Komandorskiy I.) and 20 to 22° in the northwest part of the Pacific Ocean.

The total number of bacteria was determined on "synpor" membrane filters with pore diameter of 0.2 μm. The number of individual groups of microorganisms was determined by the cup method in the following media: MPA (heterotrophs), Tiller's medium (oligocapnophils), Chapek medium with 1% diesel fuel (hydrocarbon-oxidizing + hydrocarbon-resistant). SGO with diesel fuel was inoculated in silica gel medium to calculate the hydrocarbon-oxidizing microflora from the microorganisms separated in the Chapek medium with diesel fuel (Walker and Colwell 1976). Carbon tetrachloride extraction with subsequent measurement in an "oil-102" device (Japan) was used to determine the hydrocarbons in the water samples. Krasil'nikov and Berg detectors and some original works were used to determine the species affiliation of the hydrocarbon-oxidizing microorganisms.

NUMBER AND DISTRIBUTION OF PETROLEUM-OXIDIZING AND OTHER MICROORGANISMS IN THE OCEAN

During the Arctic expedition of 1974, the northern seas were examined along the routes from Arkhangel to the Tiks Bay. The level of pollution could be described as moderate in the open waters and critical in ports and local spots in the straits. The number of microorganisms in the surface waters, counted in agar medium containing a petroleum product, consisted of 1500-7000 cells/ml. In the relatively shallow areas (9-39 m), as the depth increased and in the subsurface layer, there were one to two orders of magnitude fewer microorganisms. The number of petroleum-oxidizing microorganisms in the deep-water regions (129-208 m) on the surface and subsurface layer was 1 cell in 10 ml of sample. The number of petroleum-oxidizing bacteria in the surface layer of the port, Dikson, where strong pollution was observed, was 10 times greater than in the samples from the open sea. However, the same number of colonies grew in the media with diesel fuel and machine oil. Agar-treated mineral medium containing no hydrocarbons was used as the control. We found that a significant number of microorganisms grew in the control medium, often equaling the number of microorganisms in the medium containing the petroleum product. In investigating the microorganisms isolated in the medium containing the petroleum product in the laboratory, we found that only 46.4% of the strains also oxidized the petroleum product in a liquid medium; the remainder grew because of the agar and the impurities contained in it.

Using agar-treated medium containing a petroleum product in quantitatively calculating petroleum-oxidizing microflora produced results which were too high (almost two times) due to the oligocapnophilic forms. However, despite this fact, the number of petroleum-oxidizing microorganisms in the northern seas of the USSR is high, and the number is significantly higher in the chronically polluted regions. At the same time, a significant number of microorganisms which cannot actively use petroleum products but which carry high concentrations of these compounds, also live in the seas.

The results of the Arctic expedition induced us to turn our attention to the method of calculating the petroleum-oxidizing microorganisms and their quantitative relationships with microorganisms from other physiologic groups--heterotrophs and oligocapnophilic organisms. The method of saturated cultures in a liquid medium containing a petroleum product used for calculating petroleum-oxidizing microflora is not sufficiently precise and is inconvenient, particularly in conducting investigations in water with a low number of bacteria (less than 1 c/ml). As Japanese and American authors have shown, the most precise results are obtained by inoculations in silica gel medium containing a petroleum product (Seki 1973; Walker and Colwell 1976). The disadvantage of this method is based on the fact that it is methodologically difficult to prepare silica gel medium and impossible to do so in the conditions of an expedition. For this reason, in the subsequent studies, we used agar-treated medium containing a petroleum product, representing the bacteria which grew in the medium as the sum of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria, and we then inoculated the microorganisms separated in the laboratory in silica gel medium with a petroleum product and calculated the number of hydrocarbon-oxidizing microorganisms. We simultaneously developed a method for preparing silica gel which was suitable for field conditions.

The study of the quantitative relationships between petroleum-oxidizing and other microorganisms revealed the role and significance of petroleum-oxidizing microflora in the ecosystem with respect to the level of pollution. These microbiologic studies were conducted in open waters in the northwestern part of the Pacific Ocean. We investigated the following: (1) the number and distribution of saprophytic, oligocapnophilic and hydrocarbon-oxidizing bacteria between the surface film and the subsurface water; (2) the activity and resistance of bacterioneustons and bacterioplankton to petroleum products; and (3) the indicative significance of hydrocarbon-oxidizing bacteria.

The number of bacteria from all of the groups studied was higher in the surface film than at the meter level (Table 1). Saprophytes predominated in both levels. The number of saprophytes in the surface microlevel varied from 70 to 5500 cells per 100 ml of water; it did not exceed 500 c/100 ml at five stations, and was over 1000 c/100 ml at the seven others. The enrichment factor E* at the six stations did not exceed 10 and was greater than 10 at only three stations; the maximum value was 59.5. A higher number of saprophytic bacteria was observed on the meter level at three stations. The number of saprophytic bacteria on this level varied from 10 to 3100 c/100 ml and exceeded 1000 c/100 ml at only four stations. A correlation was found between the number of this group in the surface microlayer and the water layer (r = 0.58).

---

* The ratio of the concentration of bacteria in the surface microlayer to the concentration on the meter level.

TABLE 1. MEAN NUMBER (M) OF BACTERIA FROM DIFFERENT GROUPS AND ENRICHMENT FACTOR (E) AT STATIONS IN THE NORTHWEST PACIFIC OCEAN

Station No.	Level	Saprophytes M (c/100 ml)	Saprophytes log M	Saprophytes E	Oligocarbophils M (c/100 ml)	Oligocarbophils log M	Oligocarbophils E	HC-oxidizing+HC-resistant M (c/100 ml)	HC-oxidizing+HC-resistant log M	HC-oxidizing+HC-resistant E
1	220 µm	1350	3.13	0.6	200	2.30	2.0	60	1.78	1.2
	1 m	205	2.31		100	2.00		50	1.70	
2	220 µm	103	2.01	0.5	85	1.93	21.3	11	1.04	0.6
	1 m	220	2.34		4	0.60		20	1.30	
3	220 µm	6200	3.79	12.4	2000	3.30	500.0	1000	3.00	47.6
	1 m	500	2.70		4	0.60		21	1.32	
4	220 µm	1290	3.11	2.6	116	2.06	116.0	255	2.41	255.0
	1 m	550	2.74		1	0.00		1	0.00	
5	220 µm	55000	4.74	59.5	340	2.53	1.9	850	2.93	3.2
	1 m	925	2.97		180	2.26		265	2.42	
6	220 µm	1900	3.28	1.5	500	2.70	0.5	570	2.76	0.4
	1 m	1300	3.11		1000	3.00		1400	3.15	
7	220 µm	24000	4.38	22.9	14000	3.15	200.0	6450	3.81	71.7
	1 m	1050	3.02		70	1.85		90	1.96	
8	220 µm	300	2.48	1.3	440	2.64	9.4	245	2.39	1.5
	1 m	230	2.36		47	1.67		160	2.21	
9	220 µm	425	2.63	0.3	620	2.79	1.7	250	2.40	0.2
	1 m	1600	3.20		360	2.56		1605	3.21	
10	220 µm	3900	3.59	1.3	3800	3.58	4.8	2200	3.34	1.1
	1 m	3100	3.49		800	2.90		2100	3.32	
11	220 µm	115	2.06	1.2	40	1.60	20.0	60	1.78	15.0
	1 m	100	2.00		2	0.30		4	0.70	
12	220 µm	70	1.85	0.2	1	0.00	0.0	4	0.60	0.1
	1 m	355	2.55		1000	3.00		30	1.49	

The number of oligocarbophilic bacteria in the surface film exceeded the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria at seven stations and varied within the limits of 1 to 14,000 c/100 ml; however, the number of bacteria from this group exceeded 1000 c/100 ml at only three stations. The enrichment factor did not exceed 10 at five stations, and varied from 20 to 500 at five stations. A higher number was observed at the meter level at two stations. The number of this group of bacteria in the meter level did not exceed 100 c/100 ml at seven stations; there were 1-4 c/100 ml at four stations, and the number varied from 180 to 1000 c/100 ml at the other five stations. No correlation was found between the number of oligocapnophilic bacteria at the two levels studied.

The number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria in the surface film varied from 4 to 1000 c/100 ml at the ten stations, and reached 2200 and 6450 c/100 ml at only two stations. At the meter level, the number was 100 c/100 ml at seven stations, and varied from 160 to 2100 at the other five. The enrichment factor for the microsurface level did not exceed 3.2 at four stations, and varied from 15 to 71.7 elsewhere; the factor was 255 at only one station. A high number of bacteria was found at the meter level at only four stations. No correlation was found between the number of bacteria in the surface film and at the meter level; this indicates that there is no direct correlation between the number of bacteria in these two levels.

Overall, the mean values for the number of different bacteria in the surface film were (per 100 ml): saprophytes 7888 c, oligocapnophilic 1845 c, hydrocarbon-oxidizing + hydrocarbon-resistant 996 c; the values for the meter level were, respectively: 845, 297, and 479 cells.

A correlation was found between the number of all of the groups of bacteria studied both in the surface film and on the meter level (Table 2). The data obtained concerning the high number of saprophytic bacteria in the surface film in comparison to the meter level generally agree with the data in the literature.

However, an inverse correlation was sometimes found; this could be due to the presence of substances in the surface film which inhibit the development of bacteria (Sieburth 1972), and to a combination of other unfavorable factors--intensive solar radiation, high surface tension, high oxidation-reduction potential, hydrologic conditions, etc. The result of this could be a decrease in the number of bacterioneustons; as a consequence, accumulation of bacteria in the surface film would not adequately replace the cells from dead microorganisms (Dietz et al. 1976).

There was a close correlation between the number of hydrocarbon-oxidizing and oligocapnophilic bacteria and also between the hydrocarbon-oxidizing bacteria and the saprophytes; the relatively high correlation coefficient is an indication of this (Table 2). This apparently indicates that representatives of both the saprophytes and the oligocapnophilic bacteria are included in the composition of the hydrocarbon-oxidizing and hydrocarbon-resistant bacteria, and a closer correlation between this group and the number of oligocapnophilic bacteria was observed on the meter level.

TABLE 2. CORRELATION COEFFICIENT FOR THE NUMBER OF BACTERIA FROM THE DIFFERENT PHYSIOLOGIC GROUPS

		Level	
	Physiologic Group	220 µm	1m
1.	Oligocapnophilic - saprophytes	0.67	0.59
2.	Hydrocarbon-oxidizing and hydrocarbon-resistant saprophytes	0.85	0.70
3.	Hydrocarbon-oxidizing and hydrocarbon-resistant oligocapnophilic	0.82	0.84

The hydrocarbon-oxidizing and hydrocarbon-resistant bacteria at seven of the 12 stations represented a high percentage of the total number of the heterotrophic population at the meter level (Table 3). This indicated that the biochemical activity of the bacteria in the surface film is no higher than the activity of the bacteria in the water layer with respect to petroleum products.

TABLE 3. NUMBER OF HYDROCARBON-OXIDIZING AND HYDROCARBON-RESISTANT BACTERIA IN PERCENTAGES OF THE TOTAL NUMBER OF HETEROTROPHIC BACTERIA

	Station No.											
Level	1	2	3	4	5	6	7	8	9	10	11	12
Surface layer 220 µm	3.9	5.9	12.2	18.3	1.5	23.8	17.0	33.1	23.9	28.6	38.7	5.6
Meter layer	16.4	8.9	4.0	0.2	24.0	60.9	8.0	57.8	81.9	53.8	3.9	1.9

The highest quantity of hydrocarbons was contained in the surface layer (Table 4): from 0.38 to 3.22 mg/liter.

The concentrations of hydrocarbons on the meter level were significantly lower: from 0.06 to 0.19 mg/liter. The indicative significance of petroleum-oxidizing bacteria has been discussed in the literature.

TABLE 4. CONCENTRATION OF HYDROCARBONS IN SAMPLES OF WATER FROM THE NORTHWEST PACIFIC OCEAN (in mg/liter)

	Station No.											
Level	1	2	3	4	5	6	7	8	9	10	11	12
220 μm	3.22	2.94	3.22	1.62	1.94	1.74	0.80	0.88	1.06	0.48	0.72	0.38
1 m	0.19	0.14	0.06	0.64	0.10	0.18	0.14	0.15	0.12	0.08	0.18	0.15

This concerns whether or not it is possible to determine the degree of pollution based on the number of hydrocarbon-oxidizing bacteria. As our data indicate, no correlations between the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria and between the fractions of these bacteria in the entire heterotrophic population and the concentration of hydrocarbons were found for the surface film or for the meter level of open waters (Figures 2, 3).

The presence of bacteria which could grow in hydrocarbons and mixtures of hydrocarbons in the water samples were not correlated with the concentration of hydrocarbons in these samples. Walker and Colwell (1976) arrived at a similar conclusion in conducting studies in the Chesapeake Straits. This conclusion is logical if we consider studies of hydrocarbon absorption by the cells of microorganisms. It has been found that oxidation of hydrocarbons by microorganisms takes place when the cells are in direct contact with the substrate; the hydrocarbon enters the cell by passive diffusion and is solubilized in the lipophilic parts of the cell wall (Koronelli 1979). The concentration of the hydrocarbon in the medium should not be less than 0.1 g/liter for this process to take place. The quantities of petroleum products usually observed in open waters are significantly lower than the "substrate" concentrations; this explains the absence of any correlation between their concentration and the number of petroleum-oxidizing microorganisms. The necessary "substrate" concentrations usually occur in chronically and critically polluted bodies of water (ports, petroleum products and discharge sites, accidental discharges of petroleum). However, it is necessary to remember that the number of hydrocarbon-oxidizing bacteria does not necessarily increase proportionally at such sites, since their development will be limited by a number of other factors, primarily a shortage of biogenic elements--nitrogen and phosphorus.

We conducted studies in the northern part of the Pacific Ocean in the area of Mednyy Island to investigate the distribution of hydrocarbon-oxidizing microflora in unpolluted waters. This island, which is part of the Komandorskiy Archipelago, is one of the few places where the effect of human activity on the surrounding environment is almost absent. There are not permanent settlements on the island, and the marine freight routes are far away. For this reason, the coastal waters can be considered an unpolluted ecosystem.

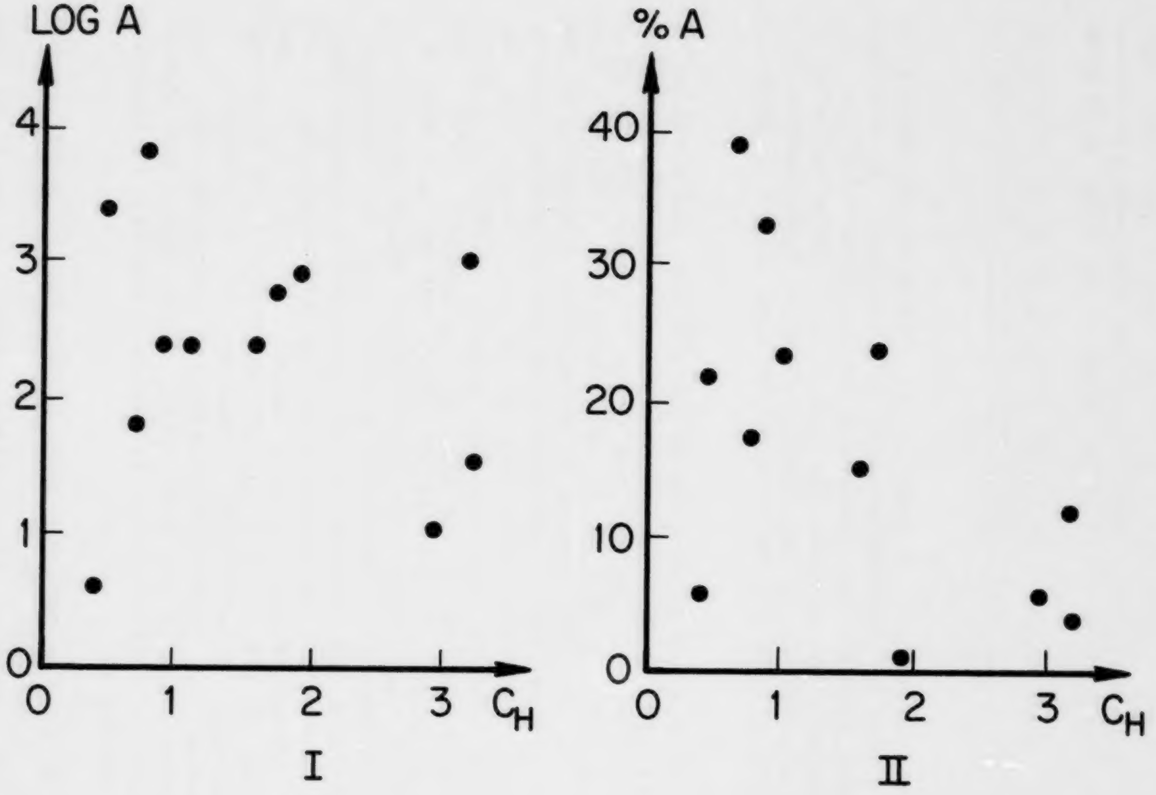


Figure 2. Correlation of the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria (I) and their percentage of the total heterotrophic population (II) with the amount of hydrocarbons in the surface film;

log A - Logarithm of the number of bacteria,
%A - percentage of the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria in the total heterotrophic population,
C_h - concentration of hydrocarbons in mg/liter.

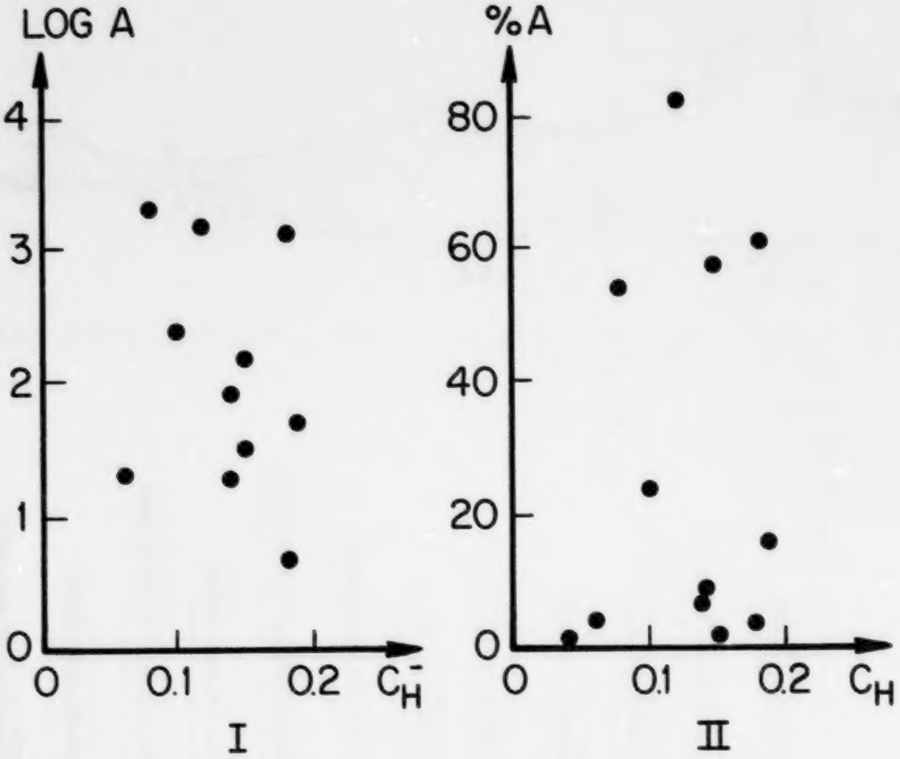


Figure 3. Correlation of the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria (I) and their percentage of the total heterotrophic population (II) with the amount of hydrocarbons at the meter level; symbols same as for Figure 2.

Although all of the island systems in the North Pacific share many common features, each one has its own characteristics which determine not only the topography and climate, but also the communities which populate them. In particular, there are large lairs of marine animals on Mednyy Island--seals and dotterel. Rich populations of seaweed have developed in the coastal waters, forming dense overgrowth in some places.

The microbiologic studies in the coastal waters off Mednyy Island were conducted during the Northern Expedition of the Biological Faculty of MSU in June-July 1976. The total number of microorganisms and the number of individual groups--heterotrophic, oligocapnophilic and hydrocarbon-oxidizing microorganisms were determined. The microbiologic studies also included both the western and eastern coasts of the southeastern part of Mednyy Island; there were 23 stations (Figure 4). The total number of bacteria varied within the limits of 10-15 million c/ml. Oligocapnophilic bacteria were most prolific; the number at the different stations varied from 120 to 24,000 c/ml, and they were predominant at 12 out of 23 stations (Figure 5).

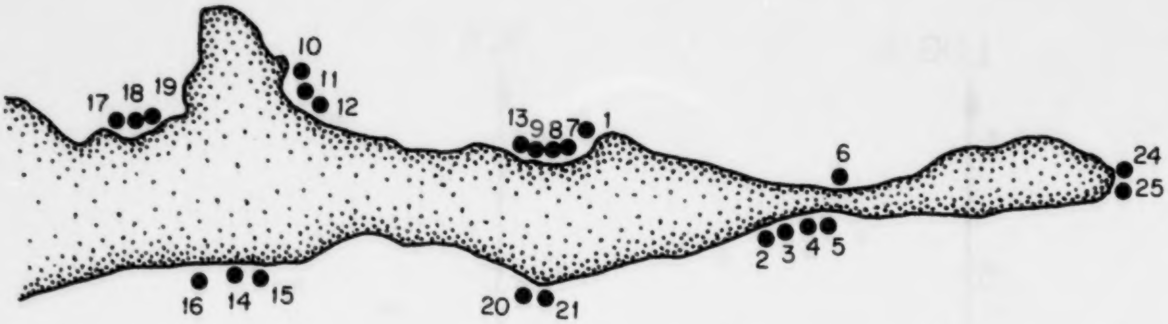

17
18
19
10
11
12
13
9
8
7
1
6
24
25
2
3
4
5
16
14
15
20
21

Figure 4. Southeastern part of Mednyy Island. *Stations where samples were taken.

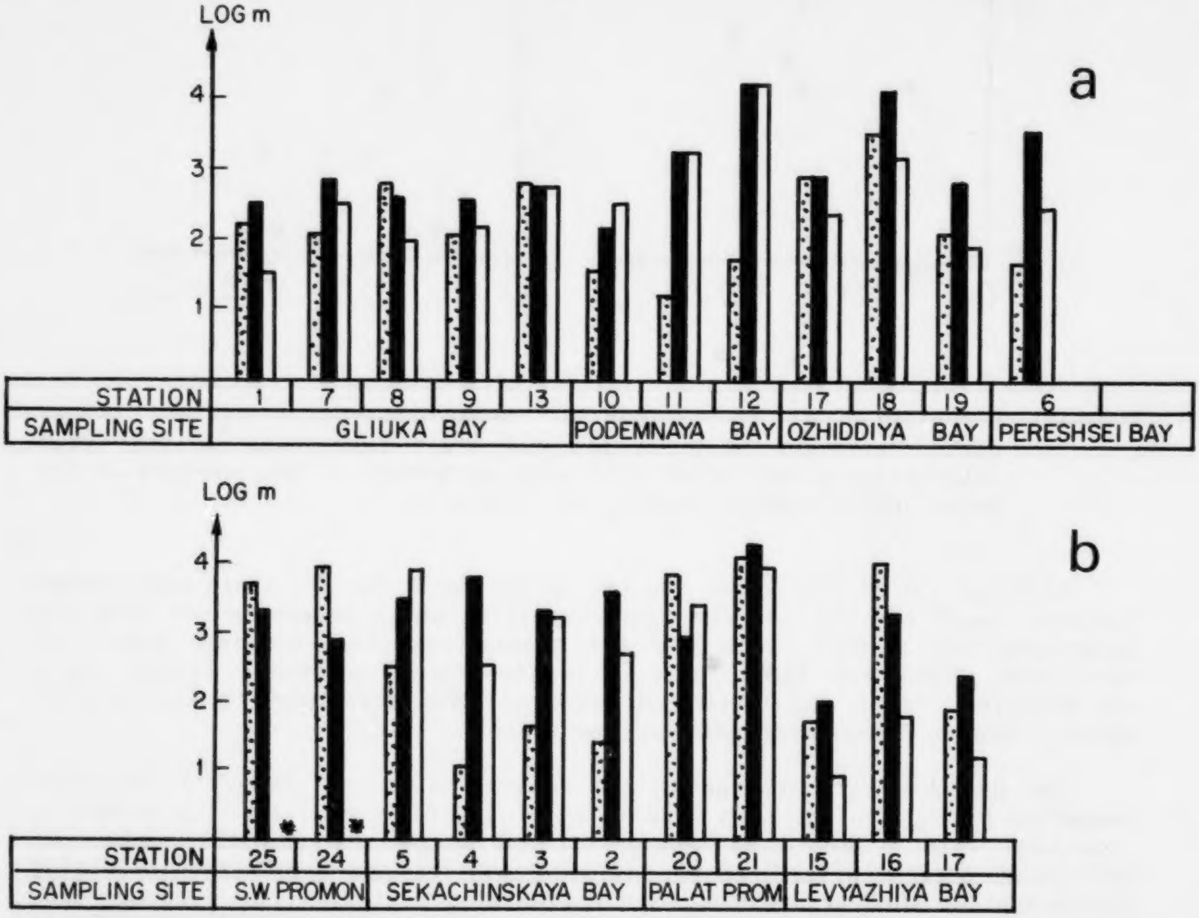


Figure 5. Number of bacteria in the coastal waters of the southeastern part of Mednyy Island: a) east coast; b) west coast. Number of bacteria (c/ml) □ hydrocarbon-oxidizing and hydrocarbon-resistant; ▨ saprophytes; ■ oligocapnophilic; *number of bacteria less than 1 c/ml.

The number of heterotrophs was generally small and did not exceed 200 c/ml at 13 stations; this indicated a very insignificant concentration of the easily accessible organic substance in coastal waters. On the western coast of the island, the number of heterotrophs was considerably higher than on the east coast: It was over 5000 c/ml at 5 out of 11 stations. It was particularly prolific at the stations near the seal and dotterel lairs (stations 24 and 25 at the Southeast Cape and stations 20 and 21 at the Palat Cape). This was probably due to the enrichment of the coastal waters by organic substances as a result of the vital activities of these animals. Studying the number of hydrocarbon-oxidizing bacteria was of more interest. No petroleum hydrocarbons were found on analysis of the samples, although the sensitivity of the recording device was relatively high--0.05 mg/liter. Nevertheless, in using the method of counting in agar-treated medium containing diesel fuel, we obtained high values for the number of hydrocarbon-oxidizing and hydrocarbon-resistant bacteria: up to 20,000 c/ml at some stations and below 200 c/ml at only eight stations. However, only 50% of the strains isolated grew in the silica gel medium. This was also true of the real hydrocarbon-oxidizing bacteria. The remaining bacteria isolated in the agar-treated medium containing a petroleum product did not assimilate the petroleum product and grew because of the organic substances contained in the agar. In analyzing the data presented in Figure 5, we see that the number of hydrocarbon-oxidizing bacteria isolated in the agar medium correlate with the number of oligocapnophilic bacteria in the overwhelming majority of the cases. A correlation between the hydrocarbon-oxidizing and heterotrophic bacteria was observed less often and only when there was a large number of heterotrophs.

However, the number of hydrocarbon-oxidizing bacteria at a number of stations remained significant and was in no way related to pollution. The presence of these microorganisms in unpolluted coastal waters is due to the variety of their food requirements, since we know that there are no highly specialized forms in this group of organisms (Shlegel' 1972). The high concentration of hydrocarbon-oxidizing bacteria at some stations could be due to the following causes. It has been shown that a large part of microbial enzyme systems responsible for oxidizing hydrocarbons from the paraffin series are inducers (Rozanova 1975; Klug and Marcovetz 1971). Oxidized hydrocarbons, the higher alcohols, aldehydes, and acids can be inductors. The formation of lipid substances of this type could be related to the vital activity of the seals and is very highly probable in this region. In addition, the copious seaweed in the coastal waters of the island could also be a source of hydrocarbons and their derivatives (Caparello and LaRock 1975). Hydrocarbon-oxidizing bacteria are thus a normal component of the coastal waters of Mednyy Island, and the large amount found at a number of stations is not an indication of pollution from petroleum products.

Similar results were recently obtained in foreign studies of microflora in the estuary of the Neuse River (North Carolina). It was found that a large number of microorganisms capable of using hydrocarbons could be found even in natural waters with comparatively low levels of pollution from petroleum hydrocarbons ($5\text{-}79 \cdot 10^{-9}$ g/ml) (Buckley *et al*. 1976).

With respect to the results of the expedition's studies, it is necessary to mention that the presence of petroleum-oxidizing microorganisms in regions moderately polluted by petroleum and in unpolluted regions is not related to the presence of petroleum products and consequently these microorganisms cannot be used as a pollution indicator in these conditions.

PETROLEUM-OXIDIZING MICROFLORA IN MARINE ECOSYSTEMS

Microorganisms which assimilate petroleum products were isolated in pure cultures and studied, and the most active forms were determined by species. Pure cultures were obtained by inoculating water samples in agar-treated medium containing diesel fuel or liquid paraffin or by the cumulative culture method in medium containing 1% paraffin (a natural source of hydrocarbons and energy). Petroleum-oxidizing hydrocarbons from the following regions were investigated: the coastal waters of the Finnish straits, Arctic Seas from Arkhangel to the Tiks Bay, the coastal waters of Wrangel I. and the Komandorskiy Islands. All of these places were in communication and were located in regions with cold and very cold climates (55-75°N latitute). The waters in the Finnish and Yenisey Straits are characterized by pronounced distillation. The concentration of salts in these regions varied from 2.3 to 37.5 g/liter.

As noted above, in inoculating the water samples in the agar-treated medium containing a petroleum product, a significant number of microorganisms which weakly assimilated or did not assimilate hydrocarbons grew in the medium. Most of them lost their capacity to grow in the medium with the hydrocarbons after a series of repeated inoculations. These bacteria are apparently either totally incapable of assimilating hydrocarbons and grow because of the organic substances in the agar, or are capable of oxidizing the hydrocarbons in special conditions. In contrast to this, the cumulative culture method made it possible to distinguish the active forms immediately.

The study of the cultural, morphologic and physiologic-biochemical properties showed that microorganisms with a stable capacity to oxidize aliphatic hydrocarbons and petroleum products are basically arthrobacteria and saprophytic mycobacteria. Hydrocarbon-oxidizing actinomycetes and fungi were found in an insignificant number. Mobile forms were only found in freshly collected materials; they usually grew weakly in the medium containing diesel fuel (a natural source of hydrocarbons and energy) and died after several inoculations. No mobile forms were found when the cumulative culture method was used.

Petroleum-oxidizing arthrobacteria were widely distributed in all of the regions examined and represented approximately half of the active microflora. They all belonged to the A. ceroformans species (old name: Mycobacterium ceroformans) described by Krasil'nikov et al. (1971). The characteristic features include: active growth in a medium containing paraffin and formation of colorless colonies which fluoresce in transient light, formation of filiform cells which rapidly decompose into short bacilli and cocci in MPA medium + 7% glycerin, gram-variability, weak growth in media containing sugar, formation of large amounts of wax when grown in media containing hydrocarbons $>C_{14}$, absence of mycolic acids; the cells are typically oval or coccal in shape and 1.0-0.8 x 0.8 μm in size.

The saprophytic mycobacteria were determined according to Krasil'nikov (1949). They all went through the bacillus → coccus → bacillus cycle in developing. The Mycobacterium mucosum species was widely distributed, and representatives of this species were found in all of the regions investigated. The related species, Mycobacterium convolutum and M. planum, were respectively isolated from water samples taken in the coastal waters of the Finnish straits and the Komandorskiy Islands. The brightly stained forms are similar to the species M. phlei and M. brevicale. Both species were encountered in all examinations of the Arctic Seas, the latter species was also found in the region of the Komandorskiy Islands. According to other classifications, the saprophytic mycobacteria described belong to the group of Coryneform bacteria, or Rhodococcus.

Arthrobacteria and mycobacteria thus play a leading role in the oxidation of petroleum hydrocarbons in the northern seas. All of the petroleum-oxidizing bacteria isolated could develop at temperatures below 10°, but to different degrees. All grew well in both fresh and salt media. In laboratory conditions, these microorganisms actively oxidized a petroleum product, and the presence of 3% sodium chloride did not interfere in this process (Table 5).

TABLE 5. GROWTH OF ACTIVE PETROLEUM-OXIDIZING BACTERIA WITH VARIED SALINITY

	Biomass, g/liter			
	Medium with diesel fuel		Medium with petroleum	
Strain	Without NaCl	+3% NaCl	Without NaCl	+3% NaCl
Mycobacterium mucosum AR-25 (5 days)	2.6	2.8	0.7	0.5
Mycobacterium phlei AR-18 (10 days)	2.6	2.8	0.8	0.4
Mycobacterium phlei AR-19 (10 days)	2.6	2.9	0.9	0.8

Growth of all strains was significantly weaker in the medium containing petroleum, and salinity had a negative effect in this case. In the flasks where strain AR-25 developed, the petroleum film disappeared in the fresh water after 2 days, and after 4 days in the salt water; in the flasks containing strains AR-18 and AR-19, it disappeared after 3 and 5 days, respectively.

Consumption of petroleum products by Arctic mycobacteria was investigated at low temperatures (Table 6).

TABLE 6. GROWTH OF MYCOBACTERIA IN A MEDIUM CONTAINING DIESEL FUEL AT LOW TEMPERATURES (growth--20 days)

Organism	Culture Temperature + 1.6°	Culture Temperature + 5.2°
Mycobacterium mucosum AR-25	Strong	Strong
Mycobacterium brevicale MST-32	Medium	Strong
Mycobacterium phlei AR-18	Medium	Medium
Mycobacterium phlei AR-19	Strong	Strong

Assimilation of the petroleum product was slowed at low temperatures, but most of the strains grew well at a temperature of 5° over 20 days, and two--M. mucosum AR-25 and M. phlei AR-19--also grew at a temperature close to zero.

Mycobacteria and the forms related to them which have a high capacity to decompose petroleum products in a model medium have been found by other authors in waters which differed in climatic conditions: in the Odessa Straits of the Black Sea (Krasil'nikov et al. 1973); in the Atlantic Ocean on the coast of New Jersey (Atlas and Bartha 1972); in different regions of the Pacific Ocean (Soli and Bens 1972; Cundell and Traxler 1973). There is also an extensive literature on oxidation of aliphatic hydrocarbons and petroleum products by mycobacteria isolated from river water and soil. These include representatives of the species which we found in the marine ecosystems. Salinity is not a significant obstacle for the development of arthrobacteria and mycobacteria in a marine environment. The data obtained indicate that the active hydrocarbon-oxidizing microflora in marine ecosystems are not specific.

CONCLUSIONS

The studies that we conducted in the northern seas of the USSR and the northwest part of the Pacific Ocean showed that microorganisms which oxidize hydrocarbons are widely distributed in these regions. They are found in particularly large quantities in places which are significantly polluted by petroleum products (port, local spills)--up to 3500 c/ml, and also in places enriched with organic matter from a non-anthropogenic origin (coastal waters in regions containing colonies of marine animals and birds, overgrowth of macrophytes)--up to 10,000 c/ml. The concentration of petroleum-oxidizing bacteria is low in open waters where no petroleum pollution is visually detectable (an average of 500 c/100 ml in the surface film) and is not correlated with the concentration of petroleum products. A large number of hydrocarbon-oxidizing bacteria always accompanies a high level of petroleum pollution, but the opposite is not always true, and a high number of these microorganisms is not an indication of pollution.

One of the most widely distributed hydrocarbon-oxidizing microorganisms is oligocapnophilic bacteria. At the same time, many marine oligocapnophilic forms cannot oxidize petroleum products, but carry high (up to 1%) concentrations in the environment. This is due to the fact that the method for counting the petroleum-oxidizing bacteria, based on using agar-treated media containing a petroleum product, produces results which are approximately twice as high. We are currently developing a method in the laboratory for preparing silica gel medium with a petroleum product which would allow counting natural petroleum-oxidizing bacteria and would be suitable for field conditions. The petroleum-degrading microorganisms isolated from sea water are arthrobacteria and saprophytic mycrobacteria (Coryneform group). The latter group includes brightly colored forms. The arthrobacteria are represented by the species Arthrobacter ceroformans; a characteristic feature of this species is the formation of large amounts of wax when liquid hydrocarbons are used. No forms specific for defined living sites were found; the same species were found in different regions. Marine petroleum-oxidizing arthrobacteria and mycobacteria are inherently euryhaline.

REFERENCES

Atlas, R. M., and R. Bartha. 1972. Degradation and mineralization of petroleum by two bacteria from coastal waters. Biotechnol. Bioeng. 14:297.

Buckley, E. N., R. B. Jonas, and F. K. Pfaender. 1976. Characterization of microbial isolates from an estuarine ecosystem: Relationship of hydrocarbon utilization to ambient hydrocarbon concentrations. Appl. and Environ. Microbiol. 32:232.

Caparello, A. M., and P. A. LaRock. 1975. A radioisotope assay for the quantification of hydrocarbon biodegradation potential in environmental samples. Microbial Ecol. 2:28.

Cundell, A. M., and R. W. Traxler. 1973. Microbial degradation of petroleum at low temperature. Marine Poll. Bull. 4:125.

Dietz, A. S., L. J. Albright, and T. Tuominen. 1976. Heterotrophic activities of bacterioneuston and bacterioplankton. Can. J. Microbiol. 22:1699.

Klug, M. J., and A. J. Markovetz. 1971. Utilization of aliphatic hydrocarbons by microorganisms. Adv. in Microbial Physiol. 5(1).

Koronelli, T. V. 1979. Absorption of hydrocarbons by microorganisms. Uspekhi mikrobiologii 14.

Krasil'nikov, N. A. 1949. Detector of bacteria and actinomycetes. Moscow, Izd-vo AN SSSR.

Krasil'nikov, N. A., L. N. Stepanova, T. V. Koronelli, and V. I. Duda. 1971. A new species of paraffin-oxidizing mycobacteria. Mikrobiologya 40:1040.

Krasil'nikov, N. A., A. V. Tsyban', and T. V. Koronelli. 1973. Uptake of normal alkanes and crude oil by marine bacteria. Okeanologiya 13:877.

Rozanova, Ye.P. 1975. The enzymatic apparatus of hydrocarbon-oxidizing microorganisms and models of mechanisms of hydrocarbon oxidation. Uspekhi mikrobiologii 10(3).

Seki, H. 1973. Silica gel medium for enumeration of petroleumlytic microorganisms in the marine environment. Appl. Microbiol. 26:318.

Shlegel', G. 1972. General Microbiology. izd. Mir, Moscow.

Sieburth, J.McN. 1972. An instance of bacterial inhibition in oceanic surface waters. Mar. Biol. 11:98.

Soli, G., and E. M. Bens. 1972. Bacteria which attack petroleum in the saline medium. Biotechnol. Bioeng. 14:219.

Walker, J. D., and R. R. Colwell. 1976. Enumeration of petroleum-degrading microorganisms. Appl. and Environ. Microbiol. 31:198.

DEVELOPMENT OF PLANKTON ALGAE IN CONDITIONS OF PETROLEUM POLLUTION

by

O. G. Mironov
Institute of Biology for Southern Seas,
AN USSR, Sevastopol'

Petroleum and petroleum products constitute one of the basic toxins which currently enter sea and fresh water. Without exaggeration, we can say that petroleum pollution is universal and the effect of other pollutants is exercised on the background of petroleum pollution of the aqueous medium. Other toxic substances often predominate in inland bodies of water--rivers, lakes, canals, etc., caused by the discharge of wastewater from different industrial enterprises. At the same time, petroleum pollution is characteristically the predominant type of pollution in oceans and seas, and marine flora and fauna primarily encounter a "pure form" of this type of pollution. The petroleum which enters the ocean is carried by currents hundreds and thousands of miles away from the discharge site, enters the subsurface layers of the water, accumulates in bottom residue, and thus affects all aspects of marine life. In this respect, the effect of petroleum on the primary production chain in the ocean is of special interest.

Galtsoff *et al*. (1936) found that the concentrations of petroleum products in ports, lagoons and bottom residue unfavorably affect diatomaceous and other algae. However, the petroleum film, in their opinion, does not kill diatomaceous algae, but only disturbs their normal fission. For example, *Nitzschia closterium* developed in an aqueous medium under a layer of different types of petroleum as well as in the controls. A 12% concentration of crude oil extracts had a stimulating effect on the growth of most diatomaceous cultures; a 25% concentration inhibited growth, and normal development of the algae stopped only with a 50% concentration. The authors concluded that both the observations under natural conditions and the laboratory data indicated that these organisms are damaged by prolonged exposure to large quantities of petroleum.

Alfimov (1956) reported that *Licmophora ehrenbergii* died 1 day after a 0.1 mg/liter concentration of solar oil was added to sea water, while the same concentration of solar oil increased the number of cells by 5 and 7.5 times in 5 days in *Melosira monifliformis* and *Grammatophora marina*. ZoBell (1964), citing a personal communication to Ruff Patrick, indicated that the petroleum film does not destroy diatomaceous algae in the underlying water, but can affect their normal reproduction.

Lacaze (1969) tested a 1% extract of crude oil of the type spilled during the TORREY CANYON catastrophe on the single-celled marine algae *Phaeodactylum tricornutum* and obtained a 10% decrease in growth. Aubert *et al*. (1969) showed experimentally that different petroleum derivatives have a harmful effect on the plankton algae *Asterionella japonica*. A 0.075 ml concentration caused the cells to die rapidly.

Fractions of several types of petroleum which are soluble in sea water and pollute the water off the coast of New York state exhibit different degrees of toxicity for *Phaeodactylum tricornutum*, *Sceletonema costatum*, *Chlorella* sp. and for the natural phytoplankton colonies in samples of sea water, according to Nuzzi's observations (1973).

Laboratory experiments with mass species of plankton and benthoplankton algae isolated from different seas were conducted in the Institute of Biology for the Southern Seas, AN USSR (Mironov and Lanskaya 1967ab, 1968, 1969; Mironov 1970; Roukhiyaynen and Mironov 1973).

As the concentration of mazut and kerosene in the sea water increased, multiplication of the algae decreased, and with a concentration of 1.0 mg/liter, the cells died in the first days. Inhibition of growth and in many cases the death of the cells were also observed with smaller concentrations of the petroleum products. Concentrations of 0.001 and 0.0001 ml/liter kerosene and mazut in sea water did not cause the algae to die during the experiment (i.e., for 5 days). No significant differences were found in the development of the experimental and control cells with these concentrations. In many cases, small quantities of petroleum products even had a stimulating effect on division of some types of phytoplankton.

In discussing the effect of petroleum pollution, we emphasize the differences in the sensitivity of the individual species. Thus, *Actinacyclus ehrenbergii* and *Hyalodiscus* sp. remained viable for 5 days with a 1.0 ml/liter concentration of mazut, while *Gymnodinium kovalevskii* and *Gymnodinium* sp. died when exposed to a 0.01 ml/liter concentration, i.e., 100 times less. Even greater differences were observed in *Ditylum brightwellii* and *Melosira moniliformis*. *D. brightwellii* died with a 0.01 ml/liter concentration of kerosene in the first days, and in 3 days with a 0.001 ml/liter concentration. At the same time, *M. moniliformis* remained viable after a 5-day exposure to sea water containing a 10.0 ml/liter concentration of kerosene. In this case, the difference in the sensitivity of the species to pollution of sea water was 3-4 orders of magnitude. Benthos and benthoplankton species were usually more resistant to pollution of the water.

Exposure of microscopic algae to different types of petroleum showed that despite slight differences in the chemical composition of the petroleum, its effect on division of plankton cells was basically a function of the concentration of the substance in the water and not a function of the type of petroleum used. A similar mechanism was observed both with species of algae which weakly divided in the experimental conditions (twice the number of cells in the control) and with intensively dividing species (5-6 and 30-fold increase in the number of cells). This apparently indicates some common feature in the effect of petroleum hydrocarbons on phytoplankton. This naturally forms no basis for suggesting that these types of petroleum have the same degree of toxicity, since this could only be determined by special studies. Kauss *et al*. (1972) showed in laboratory experiments that aqueous extracts of seven crude oils had an inhibiting effect on phytoplankton and differed in the degree of toxicity. They also observed significant changes in the pH of the medium with petroleum pollution which could inhibit growth of the algae by itself.

The differences in the species sensitivity of algae to petroleum pollution are shown in Table 1, which indicates that the cells die within a wide range of concentrations, from 1.0 to 10^{-4} ml/liter. The absence of division or inhibition of division in comparison to the controls was also observed as a function of the species of algae with a 0.1-0.00001 ml/liter concentration of petroleum. In these concentration ranges, the rate of division in a number of species of algae did not differ from the controls. Of the ten species used for the experiment, death or inhibition of cell division occurred in six species with up to 10^{-2} ml/liter concentrations of petroleum and in two species with 1.0-0.1 ml/liter concentrations. This indicates the species sensitivity of most species of plankton algae isolated from different seas to pollution of the sea water by crude oil.

Kauss *et al*. (1972) also observed a difference in the species sensitivity of phytoplankton to petroleum. This phenomenon is more evident in natural conditions, where a stimulating, indifferent, or damaging effect has been observed in plankton algae.

TABLE 1. REACTION OF ALGAE TO DIFFERENT CONCENTRATIONS OF PETROLEUM (concentrations expressed as mg/liter sea water)

Algae	Cells Died	Absence of Division or Inhibition of Cell Division	Did not Differ From Controls
Glenodinium foliaceum	1.0-0.1	0.1-0.01	0.001-0.0001
Chaetoceras curvisetus	1.0-0.01	0.01	0.001-0.0001
Gymnodinium wulffii	1.0-0.1	0.01-0.0001	--
Ditylum brightwellii	1.0-0.0001	--	--
Gymnodinium kovalevskii	1.0-0.0001	0.001-0.0001	0.00001
Prorocentrum micans	1.0	0.1-0.00001	--
Peridinium trochoideum	1.0	1.0	0.1-0.00001
Licmophora ehrenbergii	1.0	0.1-0.001	0.0001-0.00001
Platimonas viridis	1.0	0.01-0.001	0.0001
Coscinodiscus granii	1.0	1.0-0.1	0.1-0.0001

In some conditions, low concentrations of Venezuelan crude oil can stimulate photosynthesis according to the data of Gordon *et al*. (1973). On the whole, the effect of the three types of petroleum studied (Venezuelan crude, fuel oil No. 2 and fuel oil No. 6) on photosynthesis of colonies of natural phytoplankton from the basin in Bedford, New Scotland (Canada) and the northern part of the Atlantic Ocean (between Halifax and the Bermuda Islands) showed that all three toxins could inhibit photosynthesis. The current levels of petroleum pollution in the Bedford basin are capable of decreasing photosynthesis by several percent.

Intermittent contact of algae with petroleum products also results in inhibition of cell division and death of the cells. Exposing *Prorocentrum micans* and *Coscinodiscus* sp. to sea water containing 1.0 ml/liter of mazut and kerosene for one-half hour inhibited multiplication in these algae. In this case, *P. micans* was found to be highly sensitive to mazut pollution. Thus, after a 4-hour exposure to mazut, it died in 3 days in clean sea water, and died in 1 day after a 6-hour exposure. At the same time, 6-hour exposure of the same algae to the same concentration of kerosene only caused inhibition of cell division.

M. moniliformis did not lose the capacity to divide after 1-day exposure in sea water containing 10.0 ml/liter of mazut. Multiplication was not observed in the first days, however, indicating that mazut has a definite toxic effect on this alga. On the other hand, 5-minute contact of *D. brightwellii* with sea water containing 1.0 ml/liter of mazut caused statistically significant inhibition of its growth after it was placed in clean sea water. If this alga is left in sea water containing mazut for 1 hour, the cells begin to die on the third day in clean sea water.

Adding mazut and crude oil to sea water in the concentration of 0.01 ml/liter resulted in a decrease in the number of generations of *D. brightwellii* (Figure 1). Although 30% of the control cells produced ten generations, the cells exposed to the petroleum and mazut produced seven and six generations, respectively. Some cells stopped dividing in the second generation and the number of dividing cells decreased by up to 30-40% in the fourth to fifth generations.

Cell division proceeded somewhat differently in the presence of solar oil. The number of dividing cells in the experimental and control groups was initially about the same, while there were twice as many in the experimental group at the end of the experiment, i.e., in the tenth generation. The difference in the chemical composition of the petroleum products used apparently plays a role here.

Division of *Coscinodiscus granii* cells was approximately the same as division of the first species. However, due to the great resistance of *C. granii*, some of the cells generated up to ten generations in sea water containing petroleum and mazut.

Studies of the effect of petroleum, solar oil and mazut in concentrations of 0.001 ml/liter, 0.01 ml/liter and 0.1 ml/liter on the development of small Flagellata algae (pirophytic--*Cryptomonas vulgaris*, golden--*Ochromonas* sp., green--*Platymonas mediterranea*) showed (Figures 2-4) that all three species were characterized by a definite type of development both in the presence of petroleum products and in the controls. On the whole, the experiments demonstrated the clearly negative effect of petroleum products on small Flagellata algae. This was very clearly traced in *C. vulgaris* and *Ochromonas sp*.

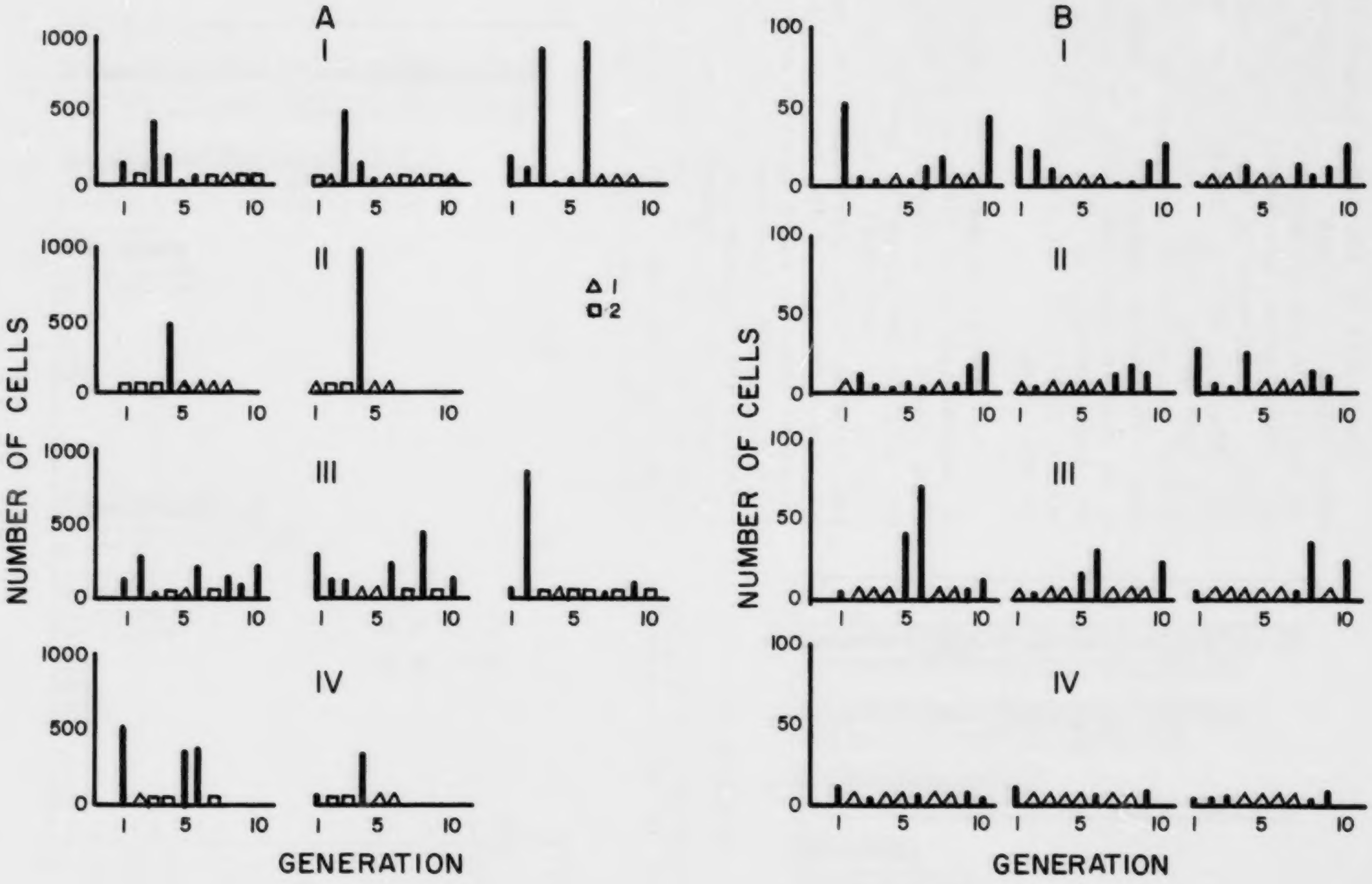


Figure 1. Maximum number of *Ditylum brightwellii* (A) and *Coscinodiscus granii* (B) cells in different generations. I-controls; II-petroleum; III-solar oil; IV-mazut; 1, single-celled; 2, under 20 cells.

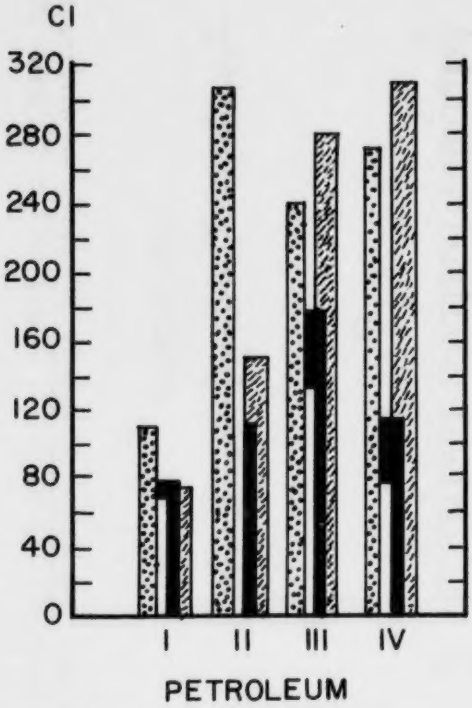


Figure 2. Development of Cryptomonas vulgaris in the presence of petroleum.

The growth of the other species of nanoplankton algae, Platymonas tetrahele, in the presence of crude oil, was investigated by Mommaerts-Billiet (1973). A decrease in the growth rate was observed with a concentration of more than 50 mg/liter. The toxicity of "fresh" and weathered petroleum was the same. The author emphasized the hazard of an even insignificant delay in growth for the species, as this could ultimately result in its elimination from the ecosystem.

The studies conducted by Kustenko on several species of Black Sea plankton algae showed their different sensitivity not only to petroleum in general, but also to its individual fractions--film and soluble. The petroleum film was most toxic for most of the species. The degree of toxicity was a function of the lighting. With continuous light, the effect of the hydrocarbons fell within the range of the optimal levels of lighting for each of the species of algae. We know that cell division in light decreases the intensity of photo-

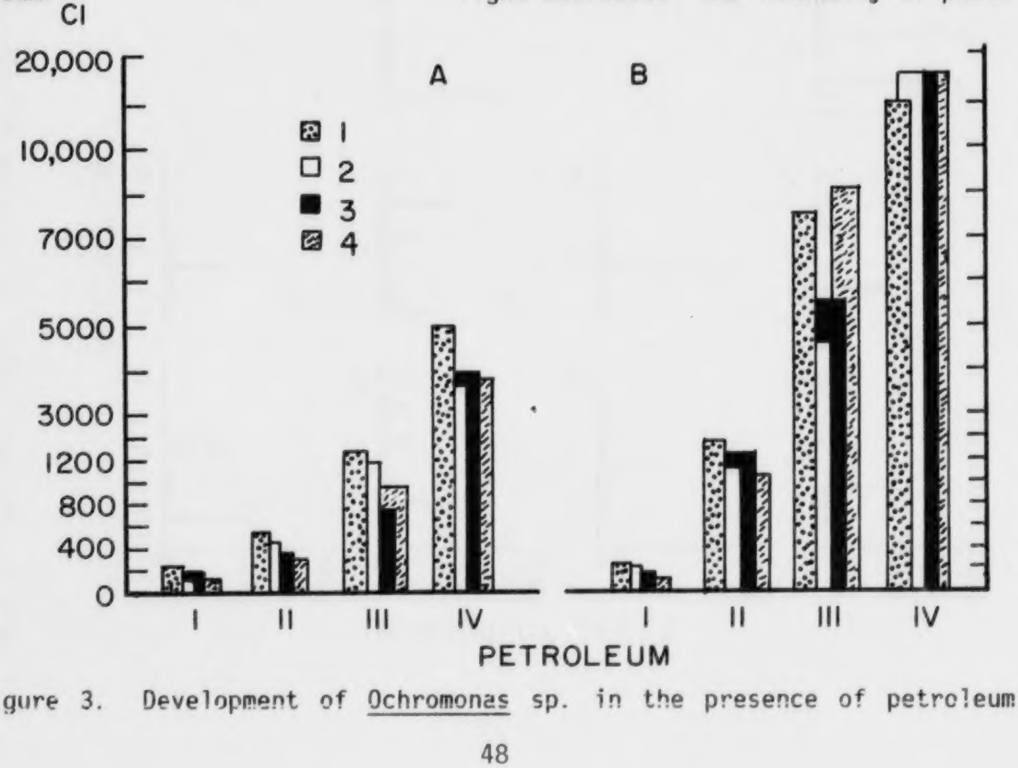


Figure 3. Development of Ochromonas sp. in the presence of petroleum.

synthesis. However, certain reserves of assimilates are even created in this case. Enrichment of the environment with oxygen also takes place. As a result, the cells can divide further and the conditions are created for multiplication of petroleum-oxidizing bacteria in aerobic conditions. For this reason, adding petroleum results in enrichment of the medium with carbon which can be utilized by the cells for treating energetic materials.

The decrease in the toxic effect of hydrocarbons observed with an 8-hour period of light could be explained in the following way: First, with this lighting regime, the rate of cell division significantly decreases. However, in this case, the light energy is apparently used more intensively by the cells than in other regimes. Nevertheless, a significant decrease in the light period does not provide sufficient reserves of assimilates for the vital activity of the cells in the remaining part of the day in conditions of total darkness. As a result, attenuation of cellular processes can begin, and the intensity with which the pollutants participate in cell metabolism decreases.

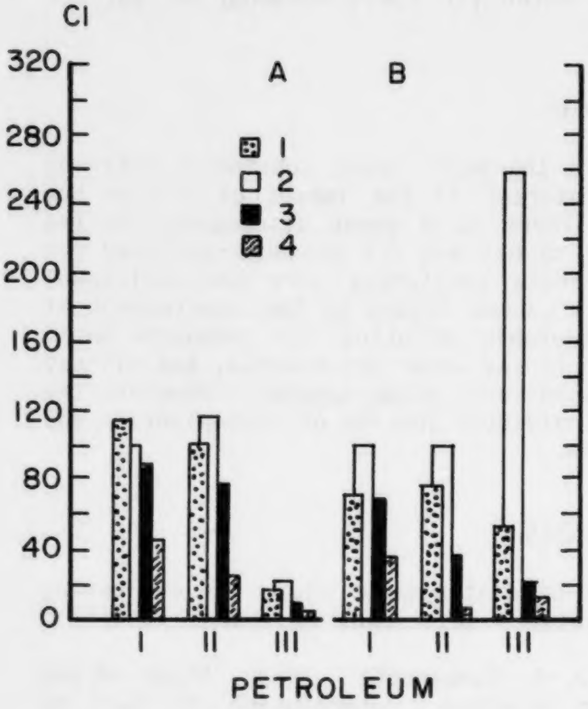


Figure 4. Development of Platymonas in the presence of petroleum.

Great differences were thus observed in the sensitivity of the individual species of microscopic algae to pollution of sea water by petroleum and petroleum products. The lethal concentrations of these substances in sea water differ by several thousand times for the individual species. Demonstrating the toxic effect of petroleum products found in sea water on plankton and benthoplankton algae isolated from different bodies of water forms the basis for hypothesizing the presence of some common mechanisms in the effect of petroleum pollution on algae.

We can hypothesize that when the water contains concentrations of petroleum products on the order of 10^{-4} to 10^{-5} ml/liter, the petroleum could sometimes significantly increase the rate of cell division in some species of microscopic algae (which can then be followed by no less significant inhibition). But the finding that individual species of phytoplankton multiply under the effect of the pollution still cannot be used as a water quality criterion.

It is very possible that small quantities of some "wastes," including hydrocarbons, in sea water result in a temporary increase in the cell division rate which can subsequently be replaced by equally pronounced inhibition, and possibly the death of the cells. The unfavorable reorganizations of the biologic structure of a given body of water whose catastrophic consequences are often observed in bodies of fresh water (in the blossoming period) are very possible.

SUMMARY

At present oil and oil products are the major toxic substances entering the marine environment. Of special interest is the impact of oil on the primary production link in the sea. There is a great discrepancy in the sensitivity of microscopic alga species to oil and oil products-polluted sea water. The lethal concentrations of these substances vary for individual species by several thousand times. Oil causes injury to the development of plankton algae generations. By considerably diluting oil products until concentrations of 10^{-4} to 10^{-5} ml/liter in sea water are reached, the oil may increase the rate of division in some microscopic algae species. However, the effect of pollution on reproduction of individual species of phytoplankton may not serve as a useful pollution criterion.

REFERENCES

Alfimov, N. N. 1956. Use of cultures of diatomaceous algae for evaluating the degree of pollution of sea water. Botanicheskiy zhurnal, 41(11).

Aubert, M., J. Aubert, S. Daniel, and J.-P. Gambarotta. 1969. Study of the effects of chemical pollution on plankton. Degradation of fuel by telluric and marine microorganisms. Rev. Int. d'Oceanogr. Med.

Galtsoff, P. S., H. F. Prytherch, R. O. Smith, and V. Koehring. 1936. Effects of crude oil pollution on oysters in Louisiana waters. Bulletin of the Bureau of Fisheries.

Gordon, D. C., and N. J. Prouse. 1973. The effects of three oils on marine phytoplankton photosynthesis. Mar. Biol. 22(4).

Kauss, P. R., T. C. Hutchinson, and M. Griffiths. 1972. Field and laboratory studies of the effects of crude oil spills on phytoplankton. Inst. Environ. Sci. and Educ. Proc. 18th Ann. Techn. Meet., New York.

Lacaze, J. C. 1969. Effects of pollution of the "Torrey Canyon" type on the unicellular marine alga Phaeodactylum tricornutum. Rev. Int. d'Oceanogr. Med.

Mironov, O. G. 1970. Division of some diatomaceous algae in sea water containing petroleum products. Biol. Nauki, 7.

Mironov, O. G., and L. A. Lanskaya. 1967a. Development of some diatomaceous algae in sea water polluted with petroleum products. In Biology and Distribution of Plankton in the Southern Seas. Moscow.

Mironov, O. G., and L. A. Lanskaya. 1967b. Effect of petroleum products on development of marine phytoplankton. In Topics in Oceanography. Naukova dumka Press, Kiev.

Mironov, O. G., and L. A. Lanskaya. 1968. Survival of some marine plankton and benthoplankton algae in sea water polluted with petroleum products. Bot. zhurn., 53(5).

Mironov, O. G., and L. A. Lanskaya. 1969. Development of marine microscopic algae in sea water polluted with hydrocarbons. In Productive-Biologic Processes in Plankton in the Southern Seas. Naukova dumka Press, Kiev.

Mommaerts-Billiet, F. 1973. Growth and toxicity tests on the marine nanoplanktonic alga Platymonas tetrathele g. s. west in the presence of crude oil and emulsifiers. Environ. Pollut. 4(4).

Roukhiyaynen, M. I., and O. G. Mironov. 1973. Development of some small marine flagellata algae in the presence of hydrocarbons. Hydrobiologic studies of the northeast part of the Black Sea. Rostov-Don, Izdat. Rostov. Univ.

ZoBell, C. E. 1964. The occurrence, effects and fate of oil polluting the sea. Adv. Wat. Pollut. Res., No. 3.

LONG-TERM BIOLOGICAL VARIABILITY AND STRESS IN COASTAL SYSTEMS

by

Robert J. Livingston
Department of Biological Science
Florida State University
Tallahassee, Florida

ABSTRACT

Coastal biological systems undergo long-term cyclic changes which are variously influenced by short-term and prolonged climatic phenomena. The biological reaction to physical stress is complex and does not necessarily follow strictly linear response patterns. This non-linearity tends to complicate the analysis of the impact of a given pollutant or pollutants. Our research group has just completed a continuous, eight-year, multi-disciplinary study of two very different bay systems in the Gulf of Mexico. This study has concentrated on long-term changes of benthic assemblages including both plant and animal species. In addition to the usual physico-chemical and biological measurements, we have concentrated on detailed trophic interactions of several fish species in an effort to understand the nature of community changes over short and long periods. Although much of the data base is still under analysis, several trends are emerging. Often such features as macrophyte standing crop and species richness of diverse groups of organisms are temporally uncoupled. Individual species strategies are diverse enough to complicate generalization based on cause and effect phenomena. The results have led us to redirect our emphasis from the taxonomic species to the "trophic unit," a group of organisms (usually a particular size class of a species) which have common feeding habits. Overall, the trophic structure of grassbed systems in the Gulf of Mexico does not follow the traditional concepts of distinct trophic levels. An understanding of the impact of habitat alteration and its effect on key units of a given trophic spectrum may be critical to the understanding of system resilience and stability. However, such an understanding will require analysis of actual overlapping successions of plant and animal assemblages which result in observed, long-term changes in coastal communities.

INTRODUCTION

The actual demonstration of cause and effect relationships due to pollution is extremely difficult because of the complexity of coastal systems and the natural variation in assemblages at various levels of biological organization. These fluctuations reflect changes in key climatic features, microhabitat distribution, water quality functions, and biological interactions such as predator-prey relationships and competition. Temporal cycles may have periods ranging from minutes to years. Despite the fact that long-term cycles with periods of many years are not only possible, but probable, most "long-term" studies of coastal systems do not exceed one year (Coull and Fleeger 1977). Rarely is enough information gathered to establish causal effects on the biological system. If specific hypotheses are to be tested regarding a system's reaction to stress, the frequency and duration of the sampling effort should be designed to fit its natural patterns of biological variability. Otherwise, the approach becomes tautological. Inadequate sampling can lead to contradiction and over-generalization. In dynamic coastal systems, there is no simple way to eliminate background variation so as to establish causal mechanisms.

Many studies have been made of long-term changes in diverse groups of coastal organisms (Longhurst et al. 1972; Williams 1972; McErlean et al. 1973; Coull and Fleeger 1977; Reid 1977; Bayley et al. 1978). However, most such studies are descriptive and without substantial insight into causal mechanisms. Coastal biological systems do not usually have a linear response to causal physico-chemical factors (Livingston et al. 1978; Meeter and Livingston 1978). The relationships of spatial variability (from system to system) and response to catastrophic natural occurrences remain poorly defined. Heavy rainfall (Boesch et al. 1976; Saloman and Naughton 1977) and temperature (Snelson and Bradley 1978) are often mentioned as important driving factors, and have been hypothesized to direct long-term trends in commercial fisheries data (Van Winkle et al. 1979; Sutcliffe et al 1977; Meeter et al. 1979). However, fisheries data are often affected by fishing effort and/or socio-economic conditions (Clark and Brown 1977; Meeter et al. 1979), and ultimately do not represent a satisfactory substitute for quantitative, multi-disciplinary, scientific data on long-term (supra-annual) changes in coastal systems.

Despite a distinct lack of actual data, there has been considerable theoretical discussion concerning the basis of stability (the ability of a system to return to normal after periods of stress or perturbation) in natural populations and communities. Orians (1974) recognized various aspects of stability, and attempts have been made to express such concepts as mathematical formations (Harrison 1979). There are numerous models which predict functional mechanisms with the usual semantic diversity (Lewontin 1969; Holling 1973; May 1973; Webster et al. 1975). That so much modeling should have been done with so little empirical testing is indeed, as McNaughton (1977) has said, "fascinating." The central problem, of course, arises from the extreme spatial and temporal variability of natural systems, especially coastal ones. The assumption that the influence of environmental changes on population variability is stochastic has been effectively questioned (Wiens 1977). Once again, from an entirely different viewpoint, the need for long-term studies is emphasized. Long-term population data (relative to generation time) is necessary because of the possibility of temporal variability in the overlapping of resource utilization traits of individual populations. At present, without these long-term data, the nature of impact due to stress (natural or anthropogenic) and system recovery remains largely undefined within the context of long-term changes in coastal systems.

AREA OF STUDY

Apalachee Bay (Figure 1) is a shallow bay system in the Gulf of Mexico which receives runoff from a series of small rivers in north Florida. It is characterized by relatively clear water (i.e. low turbidity and color), and its biological systems are dominated by benthic macrophytes which serve as a source of primary production and determine microhabitat distribution (Livingston 1974). From 1971 through 1979, a comparison was made between two of the rivers feeding Apalachee Bay, together with their offshore drainage areas: the unpolluted Econfina (Livingston 1975; Zimmerman and Livingston 1976ab, 1979) and the polluted Fenholloway. The latter received pulp mill effluents from 1954 to 1974. By changing the primary patterns of productivity and microhabitat distribution through alteration of the benthic macrophyte assemblages, the pulp mill effluents altered the trophic system drastically. During late 1974, however, a pollution-control program was initiated which eliminated considerable portions of the kraft pulp mill effluent. As a result, during the final six years of the study, we were able to study the system's capacity for return to its pre-pollution state.

METHODS

Because of similarities in water quality and flow rates to the Fenholloway River (above polluted areas), the Econfina system was chosen as a control. By comparison of the two systems, we hoped to determine the impact of pulp mill effluents on the water quality and the offshore biota of the Fenholloway, as well as to evaluate its subsequent recovery. Cognate stations were set up in the two systems (Figure 1) to facilitate statistical analysis of relative impact. Station pairs were chosen which did not differ in the value of variables not affected by the pulp mill effluents, such as salinity and temperature (Livingston 1975), so as to provide valid comparison for the evaluation of impact due to pollution (as represented by high levels of color). The data collection and analysis methods used for physio-chemical (salinity, temperature, color turbidity, pH, dissolved oxygen, Secchi depth) and biological (benthic macrophytes, epibenthic organisms) variables are given by Livingston (1975), Livingston _et_ _al_. (1976), and Zimmerman and Livingston (1976ab, 1979). The principal comparisons made were between single-station pairs and between seven-station totals of monthly data for the period of study.

RESULTS AND DISCUSSION

Long-Term Trends

Nearshore areas of Apalachee Bay are influenced by local climatic features such as temperature and rainfall. Usually, rainfall in north Florida is seasonal and major peaks occur during summer months. There are indications that upland vegetation buffers the effects of this major input of runoff through evapotranspiration, to the extent that inshore coastal systems undergo only minimal changes in water quality functions such as salinity and color. During 1973, however, there was a major rainfall during the winter and early spring period (Figure 2) which seems to have had a major influence on coastal

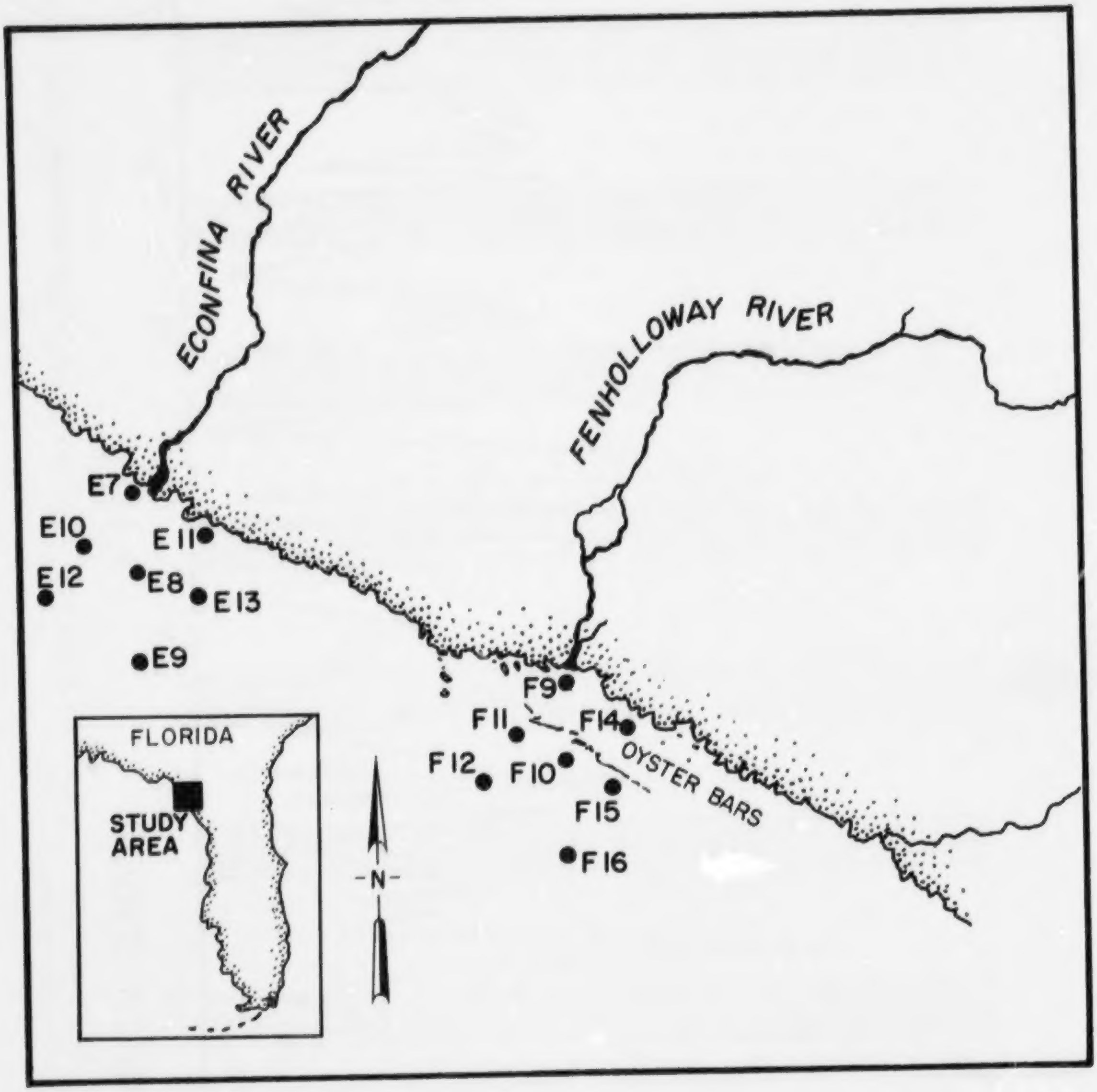

ECONFINA RIVER
FENHOLLOWAY RIVER
E7
E10
E11
E12
E8
E13
E9
F9
F11
F14
F12
F10
F15
F16
OYSTER BARS
FLORIDA
STUDY AREA
N

Figure 1. The Apalachee Bay area showing permanent sampling stations in the Econfina and Fenholloway drainage systems.

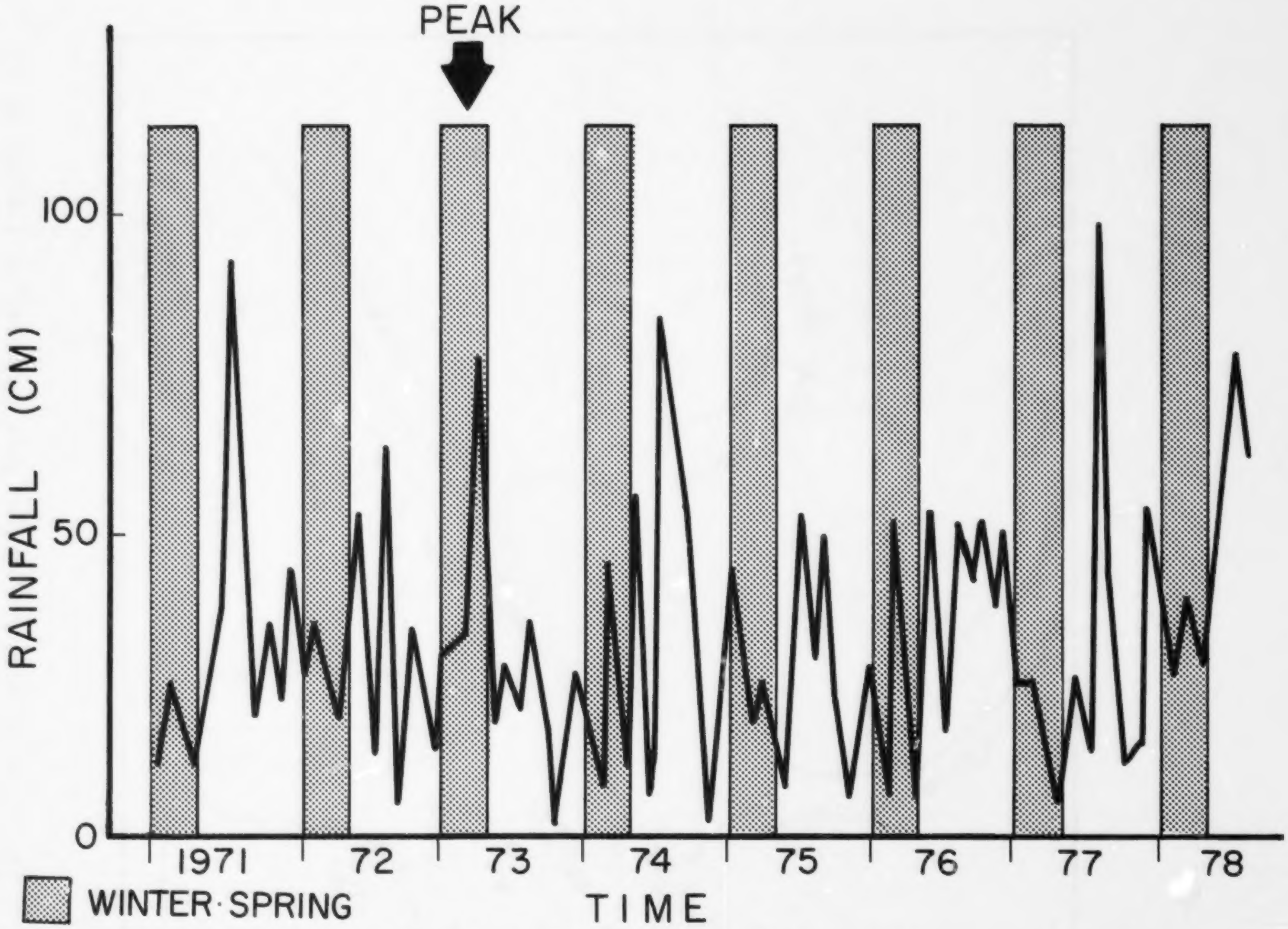


Figure 2. Total monthly rainfall (cm) in the Econfina-Fenholloway drainage area from January, 1971 through September, 1978.

water quality (Figure 3). Presumably, the (relative) dormancy of the wetlands vegetation reduced the buffering effect, resulting in the lowest salinities and the highest color levels encountered in the Econfina system during the seven-year period of sampling.

Simultaneously with the early spring rainfall of 1973, divers noted die-offs of benthic macrophytes throughout the Econfina offshore area. Macrophyte biomass remained low during the following year (Figure 4). Turtlegrass (Thalassia testudinum), the dominant macrophyte species, was particularly adversely affected by the high runoff. Recovery by this species took four years.

Long-term changes in number of macrophyte species, however, did not follow the same trends as did biomass (Figure 5). The peak in numbers of macrophyte species in the Econfina system occurred in 1974-75, the same point at which standing crops reached seven-year lows. Fish species richness in the Econfina area followed the same pattern; the peak in fish species numbers occurred during the summer of 1974. The peak in this parameter in the Fenholloway system occurred somewhat later, in 1975. The species richness curves for the two systems converged in succeeding years. Numbers of individuals, both of all fishes and of the numerically dominant species, pinfish (Lagodon rhomboides), followed the same trends as did fish species richness (Figure 6).

It is clear from these results that a single climatic shock, in the form of an unusual pattern of rainfall, can have long-lasting effects on various levels of biological organization. Because such rainfall patterns, and possibly long-term trends in winter low temperatures, show evidence of following distinct temporal patterns, an understanding of the timing of such phenomena is essential to our comprehension of the forces controlling biological variability in coastal systems.

In the present case, our long-term data base enabled us to conclude that macrophyte biomass is not coupled with species richness, either of macrophytes or of fishes. On the other hand, the close correlation between certain other long-term biological trends in both plant and animal populations seems to indicate some (direct or indirect) relationships between the controlling or modifying factors and biological response. This possibility is currently under analysis.

Trophic Structure of Fish Populations

The analysis of observed population and community changes, though important as a first estimate of impact due to stress, does not answer functional questions about dynamic interactions of various populations. The trophic structure of the system may be extremely important in the determination of population interactions and the impact of natural and anthropogenic stress on marine systems. In an attempt to understand further the nature of the changes caused by the addition of pulp mill effluents to one of the two rivers under study, a four-year study of the trophic structure of the fish assemblages in the Econfina and Fenholloway offshore systems was undertaken. The basis for this study was established by Livingston (1978). The stomach contents of the

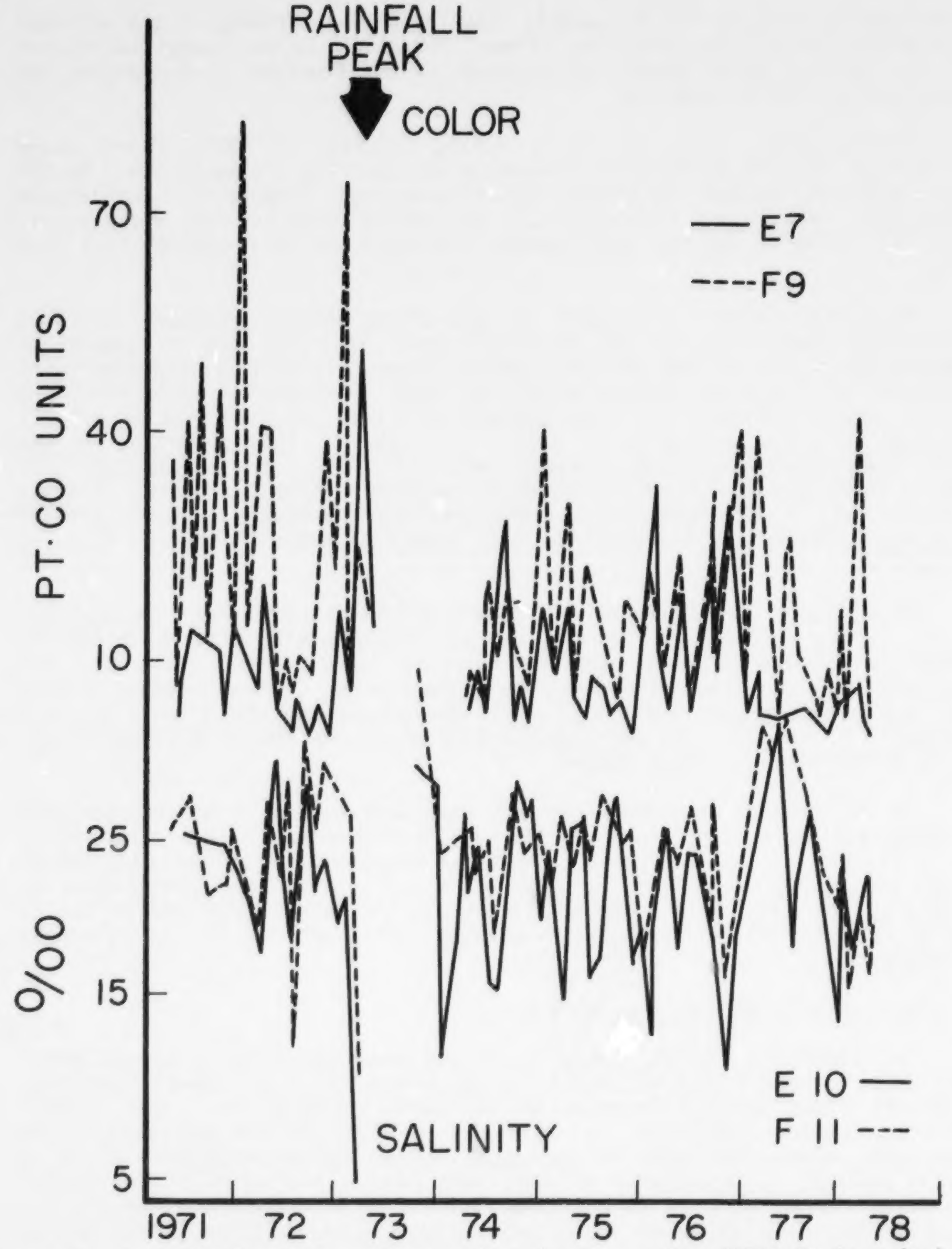


Figure 3. Salinity (parts per thousand) and water color (APHA Pt-Co units) taken monthly at stations E7 and F9 from June, 1971 through March, 1978.

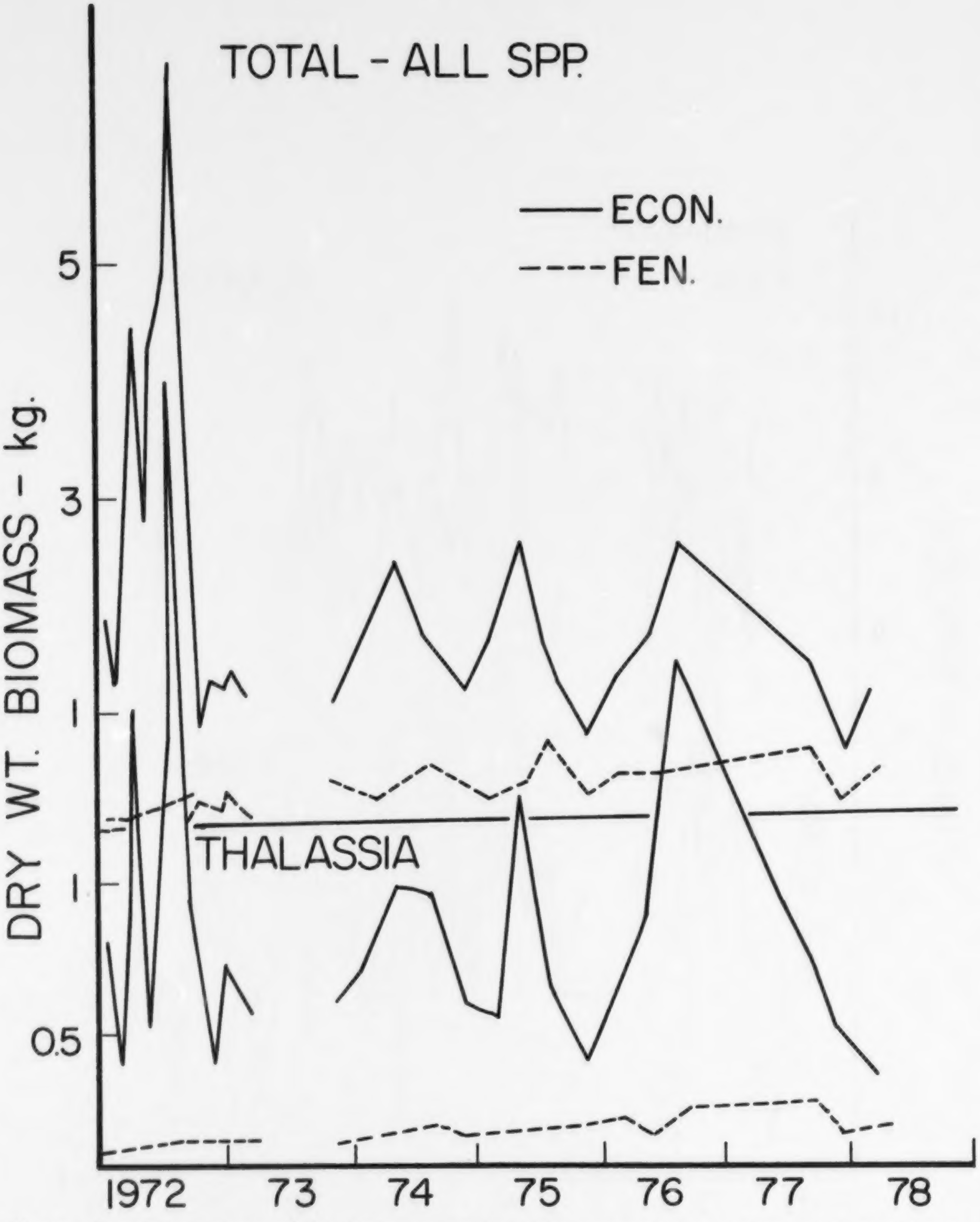


Figure 4. Total dry weight biomass of benthic macrophytes taken at 7 stations in the Econfina and Fenholloway drainage areas. Figures represent multiple samples extrapolated to biomass m^{-2} at each station. Also shown are total biomass figures for the dominant seagrass, *Thalassia testudinum*.

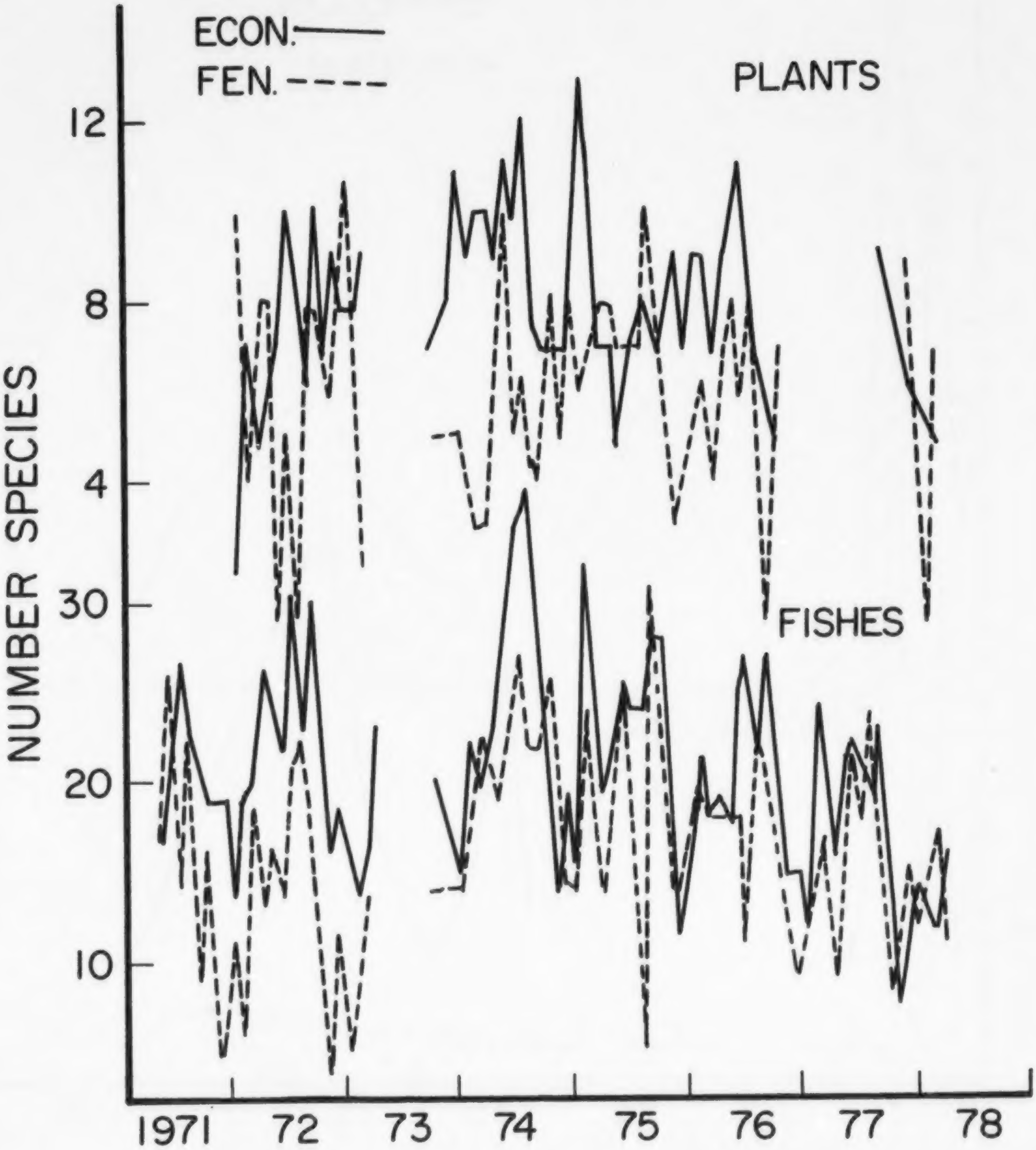


Figure 5. Total numbers of species of benthic macrophytes (gm m^{-2} at E8 and F10) and fishes (21 2-minute trawl-tows at E7, E8, E10, and F9, F10, F11) taken monthly from June, 1971 through May, 1978.

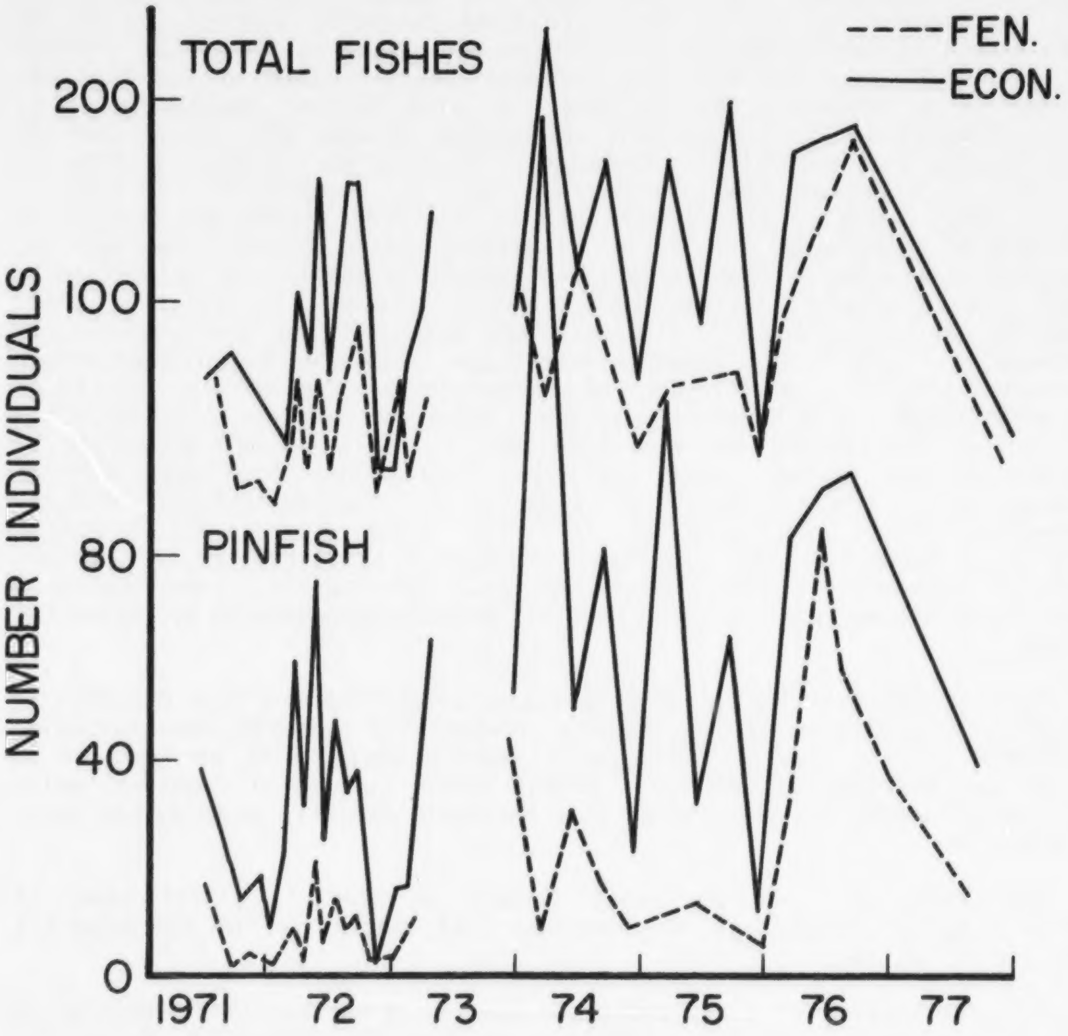


Figure 6. Total numbers of individuals of fishes taken by repetitive (49) otter-trawl tows at 7 permanent stations in the Econfina and Fenholloway offshore systems from March, 1971 through September, 1977. Also shown are total numbers of individuals of the dominant pinfish, Lagodon rhomboides.

20 numerically dominant fish species in the Econfina and Fenholloway offshore areas were identified (to species wherever possible) and quantified according to methods established by Carr and Adams (1972). The analyses included fishes taken during monthly sampling trips from 1971 through 1977 at stations chosen to allow spatial (station-specific) as well as temporal (short- and long-term) comparisons.

Because many marine organisms pass through a series of developmental stages whose ecological requirements vary considerably from one stage to the next, the species may not be the appropriate taxonomic unit to use in the establishment of the functional or causative ecological relationships. Therefore, information on the size and the developmental stage of the fish was included as a factor in the analysis. By studying the feeding habits of fishes according to species-specific ontogenetic changes and categorizing the resulting patterns by clustering techniques (Sheridan and Livingston 1979), we were able to break down the taxonomic species into a series of functional or trophic units. These units could then be analyzed within the context of considerable background information regarding basic physico-chemical and biological components of the system in an attempt to obtain some understanding of the functional basis of this shallow coastal system. The relative importance of dominance, species richness, and components of the productivity phenomena, all part of the community structure, could thus be analyzed within the context both of a spatial/temporal comparison with control stations and of long-term changes in a recovering system. Questions could be asked regarding equilibrium, resilience, and stability over a time span long enough to be relevant to the systems under analysis. Ultimately, these data will be compared to a similar series of projects in the Apalachicola estuary (Livingston 1976; Livingston _et_ _al_. 1974, 1977, 1978; Sheridan and Livingston 1979) in an attempt to test hypotheses related to spatial variability of two disparate estuarine systems (in this case the turbid, river-dominated Apalachicola estuary and the clear, benthic-macrophyte-dominated Apalachee Bay system).

The central questions regarding system stability and the relation of biological diversity to physico-chemical controlling functions are thus being approached, in the present study, on a trophic basis, with an emphasis on spatial and temporal variability. Trophic units (groups of organisms which have common feeding habits), rather than taxonomic species, serve as the basis for analysis.

The preliminary results, based largely on Stoner's (1979) study of pinfish (_Lagodon_ _rhomboides_), the dominant fish species in the Apalachee Bay area, are as follows:

1. The numbers of individuals and species of macrobenthic organisms in a given area in Apalachee Bay were directly related to the species composition of benthic macrophytes in that area. The organisms studied included suspension-feeding and carnivorous polychaetes and epifaunal amphipods and polychaetes. Deposit-feeder and omnivore numbers decreased with increased macrophyte standing crop, and seasonal changes in amphipod numbers were primarily related to reproductive mechanisms and relative abundance of fish predators.

2. There were five ontogenetic stages in the trophic composition of the pinfish, ranging from planktonic through carnivorous, omnivorous, and herbivorous phases. This succession of stages was related to morphological changes, with variation based on relative abundances of prey and macrophyte density. Selection of specific prey species was important and increased with macrophyte standing crop.

3. The key to an understanding of the trophic relationships of the fish assemblages in Apalachee Bay was the organization of benthic macrophytes in the area. There was evidence that the "trophic species" was functional as an ecological unit, casting doubt on established trophic-level approaches and the assumptions inherent in the specialist-generalist dichotomy.

SOME CONCLUSIONS

Analyses relating trophic phenomena to hypotheses concerning variability and stability of coastal systems are still being tested. However, preliminary results indicate that established theory relating impact due to stress to community structure in coastal systems may need revision. Spatially diverse estuarine systems tend to follow long-term temporal sequences of changes in species composition which reflect seasonal and supra-annual progressions. Such changes are controlled by catastrophic climatic events and long-term patterns of key physical controlling features. These cyclic phenomena have varying periodicities at different levels of biological organization and are superimposed over each other in time so that long-term aspects of community organization are complex and dynamic. Specific response to stress is based on these relationships and an understanding of trophic organization is critical to the establishment of causal phenomena. Such responses must be viewed within the context of differential phase relationships, uncoupled biomass and species richness indices, and ontogenetic changes in species ecological roles. All such functions are influenced by specific changes in productivity, microhabitat distribution, and species strategies. The durations of the relevant climatic and biological phenomena exceed the one- to three-year periods so common in established ecological surveys. Traditional (theoretical) concepts of stability and resilience often reflect naive and faulty assumptions based on inadequate empirical data. Such speculation has led to the exalted status of simplified models and indices such as species diversity which take no account of the extent of natural variability in time and space. These models and indices often involve a tacit assumption of equilibrium, a state which has not been demonstrated to exist, and is not in fact probable. Multi-dimensional variability at different levels of biological organization is simply ignored in impact analysis and, often, the natural variation of a given system exceeds that caused by a given pollutant, masking or exaggerating its effects.

Biological relationships are not necessarily linear and there is simply too little understanding of functional mechanisms in marine systems to make quantitative comparisons and generalizations possible. This fact should not be a deterrent to further modeling and speculation, but investigators should recognize that without adequate empirical data, mere hypotheses may be advanced to the status of broad generalizations. Models which are neither applicable nor predictive are being developed without necessary background information. Consequently, there is little basis for hypothesis testing. In other words, if marine ecology and pollution biology are to reach an acceptable level of credibility, considerably more "dirty" work will have to be done on natural history and basic ecological function. Hypotheses will have to be fitted to system-specific cycles of variability and factors which are critical to the functions of a given natural system will have to be identified before adequate management can become a reality.

ACKNOWLEDGEMENTS

Data analysis was supported by EPA Program Element No. 1 BA025 under Grant No. R-803339.

REFERENCES

Bayley, S., V. D. Stotts, P. F. Springer, and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of Chesapeake Bay, 1958-1975. Estuaries 1:171-182.

Boesch, D. F., R. J. Diaz, and R. K. Virnstein. 1976. Effects of tropical storm Agnes on soft-bottom macrobenthic communities of the James and York estuaries and the lower Chesapeake Bay. Chesapeake Sci. 17:246-259.

Carr, W. E. S. and C. A. Adams. 1972. Food habits of juvenile marine fishes: evidence of the cleaning habit in the leatherjacket, Oligoplites saurus, and the spottail pinfish, Diplodus holbrooki. Fishery Bulletin 70:1111-1120.

Clark, S. H., and B. E. Brown. 1977. Changes in biomass of finfishes and squids from the Gulf of Maine to Cape Hatteras, 1963-74, as determined from research vessel survey data. Fishery Bulletin 75:1-22.

Coull, B. C., and J. W. Fleeger. 1977. Long-term temporal variation and community dynamics of meiobenthic copepods. Ecology 58:1136-1143.

Harrison, G. W. 1979. Stability under environmental stress: resistance, resilience, persistence, and variability. Amer. Nat. 113:659-669.

Holling, C. S. 1973. Resilience and stability in ecological systems. Ann. Rev. Ecol. Syst. 5:1-24.

Lewontin, R. C. 1969. The meaning of stability. In Diversity and Stability in Ecological Systems, G. M. Woodwell and H. H. Smith (eds.), 13-24.

Livingston, R. J. 1974. The ecological impact of pulp mill effluents on aquatic flora and fauna in north Florida: comparison of a polluted drainage system (Fenholloway) with an unpolluted one (Econfina). Florida Coastal Coordinating Council. 87 pp.

Livingston, R. J. 1975. Impact of kraft pulp-mill effluents on estuarine and coastal fishes in Apalachee Bay, Florida, USA. Marine Biology 32:19-48.

Livingston, R. J. 1976. Time as a factor in environmental sampling programs: diurnal and seasonal fluctuations of estuarine and coastal populations and communities. In Symposium on the Biological Monitoring of Water Ecosystems, J. Cairns, Jr. (ed.), ASTM STP 607:212-234.

Livingston, R. J. 1978. Multiple factor interactions and stress in coastal systems: a review of experimental approaches and field implications. In Marine Pollution: Functional Responses, W. B. Vernberg and F. J. Vernberg (eds.). Academic Press, N.Y.

Livingston, R. J., R. L. Iverson, R. H. Estabrook, V. E. Keys, and J. Taylor, Jr. 1974. Major features of the Apalachicola Bay system: physiography, biota, and resource management. Florida Scientist 37:245-271.

Livingston, R. J., R. S. Lloyd, and M. S. Zimmerman. 1976. Determination of adequate sample size for collections of benthic macrophytes in polluted and unpolluted coastal areas. Bull. Mar. Sci. 26:569-575.

Livingston, R. J., N. Thompson, and D. Meeter. 1978. Long-term variation of organochlorine residues and assemblages of epibenthic organisms in a shallow north Florida (USA) estuary. Marine Biology 46:355-372.

Livingston, R. J., D. C. White, and R. L. Iverson. 1977. Energy relationships and the productivity of Apalachicola Bay. Florida Sea Grant Final Report. 437 pp.

Longhurst, A., M. Colebrook, J. Gulland, R. Le Brasseur, C. Lorenzen, and P. Smith. 1972. The instability of ocean populations. New Sci. 54:500-502.

McErlean, A. J., S. G. O'Connor, J. A. Mihursky, and C. I. Gibson. 1973. Abundance, diversity, and seasonal patterns of estuarine populations. Est. Coastal Mar. Sci. 1:19-36.

McNaughton, S. J. 1977. Diversity and stability of ecological communities: A. empiricism in ecology. Amer. Nat. 111:515-525.

May, R. M. 1973. Stability and Complexity in Model Ecosystems. Princeton University Press, Princeton, N.J.

Meeter, D. A., and R. J. Livingston. 1978. Statistical methods applied to a four-year multivariate study of a Florida estuarine system. In Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses, J. Cairns, K. Dickson, and R. J. Livingston (eds.). ASTM STP 652.

Meeter, D. A., R. J. Livingston, and G. C. Woodsum. 1979. Short- and long-term hydrological cycles of the Apalachicola drainage system with application to Gulf coastal populations. In Ecological Processes in Coastal and Marine Systems, R. J. Livingston (ed.). In press.

Orians, G. H. 1974. Diversity, stability, and maturity in natural ecosystems. In Unifying Concepts in Ecology. Witt, van Dobben, and R. H. Lowe-McConnell, 139-150.

Reid, P. C. 1977. Continuous plankton records: changes in the composition and abundance of the phytoplankton of the north-eastern Atlantic Ocean and North Sea, 1958-1974. Marine Biology 40:337-339.

Saloman, C. H. and S. P. Naughton. 1977. Effects of Hurricane Eloise on the benthic fauna of Panama City Beach, Florida, USA. Marine Biology 42:357-363.

Sheridan, P. F., and R. J. Livingston. 1979. Cyclic trophic relationships of fishes in an unpolluted, river-dominated estuary in north Florida. In Ecological Processes in Coastal and Marine Systems, R. J. Livingston (ed.). In press.

Snelson, F. F., and W. K. Bradley, Jr. 1978. Mortality of fishes due to cold on the east coast of Florida, January, 1977. Florida Sci. 41:1-12.

Stoner, A. W. 1979. The feeding ecology of Lagodon rhomboides (Pisces: Sparidae) and the macrobenthos of Seagrass Meadows in Apalachee Bay, Florida. Ph.D. Dissertation, Florida State University.

Sutcliffe, W. H., Jr., K. Drinkwater, and B. S. Muir. 1977. Correlations of fish catch and environmental factors in the Gulf of Maine. J. Fish. Res. Bd Can. 34:19-30.

Van Winkle, W., B. L. Kirk, and B. W. Rust. 1979. Periodicities in Atlantic coast striped bass (Morone saxatilis) commercial fisheries data. J. Fish. Res. Bd Can. 34:54-62.

Webster, J. R., J. B. Waide, and B. C. Patten. 1975. Nutrient recycling and the stability of ecosystems. In Mineral Cycling in Southeastern Ecosystems, F. G. Howell (ed.). A.E.C. Symp. Ser. Conf. 74-0513, 1-27.

Wiens, J. A. 1977. On competition and variable environments. Amer. Sci. 65:590-597.

Williams, A. B. 1972. A ten year study of meroplankton in North Carolina estuaries: mysid shrimps. Chesapeake Sci. 13:254-262.

Zimmerman, M. S., and R. J. Livingston. 1976a. The effects of kraft mill effluents on benthic macrophyte assemblages in a shallow bay system (Apalachee Bay, north Florida, USA). Marine Biology 34:297-312.

Zimmerman, M. S., and R. J. Livingston. 1976b. Seasonality and physiochemical ranges of benthic macrophytes from a north Florida estuary (Apalachee Bay). Contr. Mar. Sci. Univ. Texas 20, 34-45.

Zimmerman, M. S., and R. J. Livingston. 1979. Dominance and distribution of benthic macrophyte assemblages in a north Florida estuary (Apalachee Bay, Florida). Bull. Mar. Sci. 29:27-40.

SOME FEATURES OF THE BIOLOGIC EFFECTS OF POLLUTANTS ON MARINE ORGANISMS

by

S. A. Patin
All-Union Fisheries and Oceanography
Scientific Research Institute
Moscow

Extensive data have been collected on the effect of pollutants on many species of marine organisms. The variety of this material, the difference in methods, procedures and experimental conditions all complicate general interpretation of the vast toxicological data base which increases every year. Several attempts have been made; the results of one will be presented in this paper.

We previously emphasized the characteristic tendency toward an increase in the intensity (coefficient) of accumulation of radioactive and chemical microcomponents in marine organisms as the size of the hydrobionts decreases and, consequently, the size of their surface and contact with the water increases (Patin 1971). If this is true, then the stable differences in the degree of accumulation of toxic microimpurities in the organisms of different sized groups should result in corresponding differences in the formation of the dose loads of the toxic substances in the biomass of the organisms in these groups and, consequently, to different effects of their reaction with the same initial concentrations of harmful substances in the water.

We used the data from the literature (over 300 published works) to test this hypothesis; a summary of the results was presented in our paper (Patin 1979). The values of the logarithms of the mean lethal concentration (LC_{50}) in 48-96-hour experiments for each of the most common toxic substances, including mercury, copper, lead, cadmium, zinc dissolved in petroleum products and organochlorine compounds, were plotted graphically as a function of the logarithm of the mean size of the hydrobionts. One of these graphs is shown in Figure 1.

An evident decrease was found in the values of the LC_{50} as the mean size of the hydrobionts representing the basic groups of the biotic population in the world's oceans decreased, including the simplest plankton and benthos, mollusks, worms, and fish in different stages of ontogenesis. The correlation coefficients for the LC_{50} and mean dimensions of the organisms were equal to

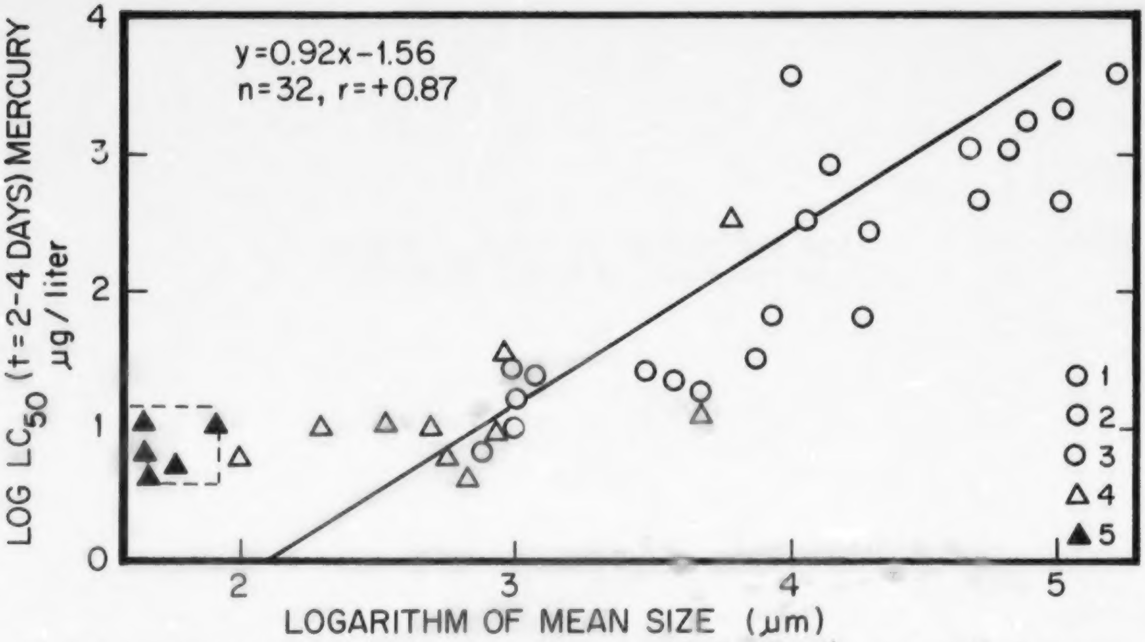


Figure 1. Correlation of the toxicity of mercury (Hg^{2+}) for marine hydrobionts with their size: 1,3-data from the literature; 2,4-data from the present study; 1,2-adult organisms; 3,4-early stages of ontogenesis; 5-value of the EC_{50} for unicellular algae; n-number of data; r-correlation coefficient; data which were not considered in calculating the regression equation are delimited by the broken line.

0.87 for mercury, 0.78 for copper, 0.88 for zinc, 0.84 for cadmium, 0.87 for lead, and 0.73 for dissolved petroleum products, 0.91 for polychlorinated biphenyls and 0.47 for all groups of organochlorine substances.

In determining these correlations, we did not consider the data relating to unicellular algae, since these data do not reflect the survival rate, as in animal experiments, but the relative change in the bioproduction indices and the cell division rate. In addition, note the correspondence of these results with respect to the general tendency toward a decrease in the resistance of hydrobionts to toxins as their dimensions decrease (Figure 1). The data on bacteria were also excluded due to the small number and the difficulty in comparing them with other materials.

The results obtained form the basis for attributing the size of hydrobionts which reflects the degree of development of the area of contact with the environment and the capacity to accumulate contaminants to one of the basic factors which predetermine the intensity and nature of toxic effects in marine ecosystems. In each concrete case, the different representatives of the marine population with the enormous variety of physiological, biochemical, morphological, trophic and other features of the existence and vital activity in different biotopes, naturally have their own specific features with respect to their reaction to any effect, including toxic effects. If we see an obvious correlation between the toxic effect and the size of the hydrobionts on this background, we must assume that the tendency is sufficiently universal and reflects objectively existing relationships and phenomena in the complex picture of the reaction of marine organisms to toxic factors in the environment.

In addition to the reasons for the low resistance of small species and forms noted above, we also note the fact that organisms in different stages of development are also included in this category: As all of the current toxicological data, including the data obtained in our laboratory indicate (Patin *et al*. 1978), these forms are significantly more sensitive than the adult specimens. The question of the nature and mechanism of this interesting phenomenon is still debated, although it would be useful for solving a number of problems in aquatic toxicology.

A particularly strong correlation between the LC_{50} and the size of the organisms is characteristic of metals, while the correlation is not as strong for dissolved petroleum products and organochlorine compounds. The reason for this probably lies in the known multicomponent nature of organic toxins and the difficulty of obtaining comparable data in conducting experiments with these substances. The variations in the experiments with organochlorine compounds, which include DDT and its metabolites, polychlorinated biphenyls, aldrin and other substances which differ in composition and properties, are particularly significant. This is the reason the correlation between the LC_{50} and size of the hydrobionts was weaker for all organochlorine compounds than for the polychlorinated biphenyls alone: The correlation coefficients were 0.47 and 0.91, respectively.

Extrapolating the experimental results and conclusions to natural conditions is difficult. Strictly as a first approximation, we can hypothesize that phytoplankton, microzooplankton filter feeders, eggs and larvae of benthos and nekton animals, especially in hyponeuston, are among the groups which are most sensitive and react most rapidly to toxic pollution. We can also hypothesize that the most rapid structural and functional disorders should arise in associations of plankton and hyponeuston where the small species and forms of hydrobionts with low resistance to toxins predominate. Similar disorders probably begin more slowly in associations of nekton and benthos where large forms predominate, but they are more stable in nature.

A more definite conclusion can be drawn with respect to the prospects for practical utilization of small species and forms of hydrobionts as test objects for experimental toxicologic control of the hazard of waste waters, their components and other substances which enter the seas. The relative simplicity and ease of working with such species (especially with unicellular algae, the simplest, smallest plankton crustaceans), the possibility of year-round cultivation, the high sensitivity and rapidity of the reaction to toxic contaminants in the environment--these and other advantages raise the hope that small species of marine organisms will be widely used in determining the danger of waste water from different enterprises and monitoring the quality of natural sea water.

SUMMARY

On the basis of data cited in scientific literature, the existence of a negative correlation was shown between the rate of sensitivity (based on 48-96 hr LC_{50}) of marine organisms of various systematic groups to the major pollutants in the marine environment (dissolved petroleum products, organo-chlorine intoxicants, mercury, lead, cadmium, copper, zinc) and the size of hydrobionts. This conclusion is of particular interest for evaluating biological damage due to pollution in the marine environment and also links theory with the practice of use of minor species and forms of hydrobionts in sewage water biotesting.

REFERENCES

Patin, S. A. 1979. Effect of pollution on biologic resources and productivity in the world's oceans. Moscow, Pishchepromizdat.

Patin, S. A., V. K. Dokholyan, N. S. Chernyshev, A. M. Akhemdov. 1978. Toxicology of some species of Caspian and Atlantic fauna. Tr. VNIRO, vol. 134.

FISH AS AN OBJECT FOR MONITORING PETROLEUM POLLUTION OF THE MARINE ENVIRONMENT

by

N. D. Mazmanidi, G. I. Kovaleva, and A. M. Kotov
Georgian Branch of the VNIRO

In monitoring pollution of the marine environment, an important place must be assigned to fish as an integral link in the trophic chain of the marine bioecosystem.

The position could be based on a number of hypotheses. First, fish are the most highly organized aquatic animals with a very differentiated nervous system (not including marine mammals, of course). Second, fish are more sensitive to toxic substances, particularly petroleum, than other aquatic organisms (Stroganov 1976; Rice et al. 1976). Third, it is not only possible to conduct analytic studies on the molecular level in fish, but also to observe all symptoms of poisoning (coordination, behavior, color, respiration, etc.).

In selecting representative organisms, we departed from the principle that experimental toxicological studies should be conducted not only for the purpose of determining admissible levels of pollution in and evaluating the quality of an aquatic medium, but also as a component part of any ecotoxicological prognosis. In this respect, the object (or objects) to be investigated should be a constituent element of the ecosystem in the region under consideration. Stating the problem in this way predetermined our approach to selecting representative species of fish for the southeastern and eastern parts of the Black Sea. For this reason, before beginning the toxicological experiments on fish, we decided to consider a series of questions:

a) Which species of fish were most common in the zone under investigation and what were the dynamics of catching them?

b) What was the basic weight-size composition of the captured specimens?

c) How would the species adapt to long periods of aquarium living?

d) How easy is it to distinguish between males and females (for correct grouping of specimens in experiments and for obtaining mature sexual products and working with them in different stages of ontogenesis)?

e) What is the sensitivity of these species to toxic chemicals?

The fish were caught with fixed nets and divided into three groups:

- Frequently found in large numbers;
- Infrequently found in small numbers;
- Individual specimens.

The representatives of the second and third groups could not be used for our toxicological studies because of the small number found. For this reason, we concentrated on the first group. It included:

- Horse mackerel - *Trachurus mediterraneus ponticus*
- Smarida - *Spicara smaris*
- Mullet - *Mullus barbatus ponticus*
- Crucian carp - *Diplodus annularis*
- Sole - *Solea lascaris nasuta*
- Plaice - *Platichthus flesus luscus*

Each of these species had to meet two important requirements: incidence and size-weight composition.

The final conclusion was drawn after orientational experiments, acute and subacute, were conducted on the survival rate and the sensitivity of these species to dissolved petroleum products had been determined.

The experiments showed that pelagic active species of smaris and horse mackerel were the most sensitive, while sole and plaice were the most resistant benthoic species. The other two species occupied an intermediate position. However, the horse mackerel did not survive in aquarium conditions. For this reason, smaris and sole were selected for the toxicological studies.

The toxicological results for smaris should be used to establish the MAC for petroleum products and the data on sole should be used for ecotoxicological prognoses.

The method used in the experiments has been described in detail in our previous studies (Mazmanidi *et al*. 1972; Mazmanidi and Kovaleva 1972; Mazmanidi *et al*. 1975). We note only that the experiments were conducted with dissolved petroleum products, and that we made certain that the experimental materials and experimental conditions were compatible. In addition to the survival rate, a number of morphological and biochemical indices for the blood, carbohydrate metabolism in the organs and tissues, and the histopathologic picture were selected as toxicity criteria. We departed from Federov's conception (1976): First, these indices to a great degree ensure homeostasis in fish, and second, changes in these indices are nonspecific response reactions to very different factors.

We emphasize that the toxicological studies were preceded by many years of work on the seasonal changes in all of the indices considered for the purpose of determining the lower and upper limits for basal data for use in interpreting the results obtained in the experiment. We also note that we concentrated on conducting chronic experiments with low concentrations of dissolved petroleum products.

When considering poisoning in fish from dissolved petroleum products, it is first necessary to discuss the symptoms of toxicosis.

Disorders in the central nervous system were observed in acute (25-29 mg/liter concentration of dissolved petroleum products) and subacute (15-19 mg/liter) experiments on active smaris. These disorders were manifested by different degrees of excitation with subsequent inhibition of motor activity.

Narcotic and paralytic symptoms predominated in the less mobile benthic fish in acute and subacute poisoning.

The investigation of the three response reactions (primary, anodic, electroshock) in an electrical current field in smaris and plaice poisoned with petroleum products confirmed the clinical symptoms: The threshold of these reactions was lower in smaris than in plaice (Mazmanidi and Balayev 1974; Balayev *et al*. 1976). Local pathological processes were simultaneously observed in the fish in acute poisoning, in addition to the CNS disorders. In addition to significant affection of the gill epithelium (hemorrhaging, edema) observed on pathological-anatomical autopsy, histological studies revealed some disorders in the structure of the organs and tissues (Mazmanidi 1974; Mazmanidi and Zambakhidze 1974).

There were also disorders in production of mucus. Hypothesizing that the skin, as well as the gills, are one of the barriers whose rupture allows petroleum poisoning to begin, we investigated the effect of dissolved petroleum products on a number of mucus indices for the skin and olfactory bulbs of smaris. In the course of petroleum toxicosis, significant changes were observed in the concentration of total protein and the ratio of protein fractions in mucus from the olfactory lining and the isoenzymatic protein fraction picture and peroxidase activity in mucus from the skin of the fish (Korolev *et al*. 1978).

Asphyxia was one of the major symptoms of acute poisoning. As our studies showed (Mazmanidi 1977), the clinical signs characteristic of this symptom were closely correlated with a sharp decrease in blood oxygen saturation (Table 1).

TABLE 1. OXYGEN SATURATION OF BLOOD IN ACUTE AND SUBACUTE POISONING BY DISSOLVED PETROLEUM PRODUCTS (in %).

Fish	N	1-10 h	24 h	48 h	72 h	96 h
Smaris	$\frac{10}{12}$	$\frac{65.5}{45.0}$	$\frac{56.5}{40.0}$	---	$\frac{52.0}{38.0}$	---
Sole	$\frac{8}{10}$	$\frac{75.0}{65.0}$	---	$\frac{72.0}{72.0}$	---	$\frac{68.0}{58.0}$

Note: The numerator indicates the controls; the denominator indicates the experimental fish; n = number of specimens examined.

As the above data indicate, this process is slightly more pronounced in the smaris. This also indicates the high sensitivity of pelagic fish to the toxic components in petroleum in comparison to benthic fish.

The decrease in blood oxygen saturation is related to a disorder in blood hemoglobin metabolism, as the blood is the principal supplier of energy for metabolic processes in fish (Table 2).

To briefly summarize the data presented in these tables, we can conclude that anoxia, clinically manifested by asphyxia and functionally by a decrease in blood oxygen saturation and disorders in the metabolsm of the different hemoglobin fractions with pronounced meth- and sulfhemoglobinemia, occupies an important position in the pathogenesis of acute and subacute poisoning from petroleum toxicoses in fish.

In reference to the morphological and biochemical blood indices, leukocytosis was observed in acute and subacute petroleum poisoning; it was within the limits of 2σ in the sole and reached 3σ in the smaris (Table 3). The same concentrations of petroleum also caused a change in the qualitative composition of the white blood, manifested by monocytopenia and lymphocytopenia; it exceeded 2σ in the smaris, and was manifested by neutrophilic leukocytosis in the sole.

The changes in the red blood cells were characterized by oligochromemia and erythropenia.

The experiments conducted with small concentrations of dissolved petroleum products showed that a 0.05 mg/liter concentration does not substantially affect the blood of benthic, less mobile marine fish (sole), while only a 0.02 mg/liter concentration is not toxic for demersal forms (smaris).

In addition to the quantitative red and white blood indices, the effect of petroleum on the qualitative composition of the blood was also studied. Acute and subacute poisoning resulted in significant disorders in the form and composition of the cellular elements in the blood.

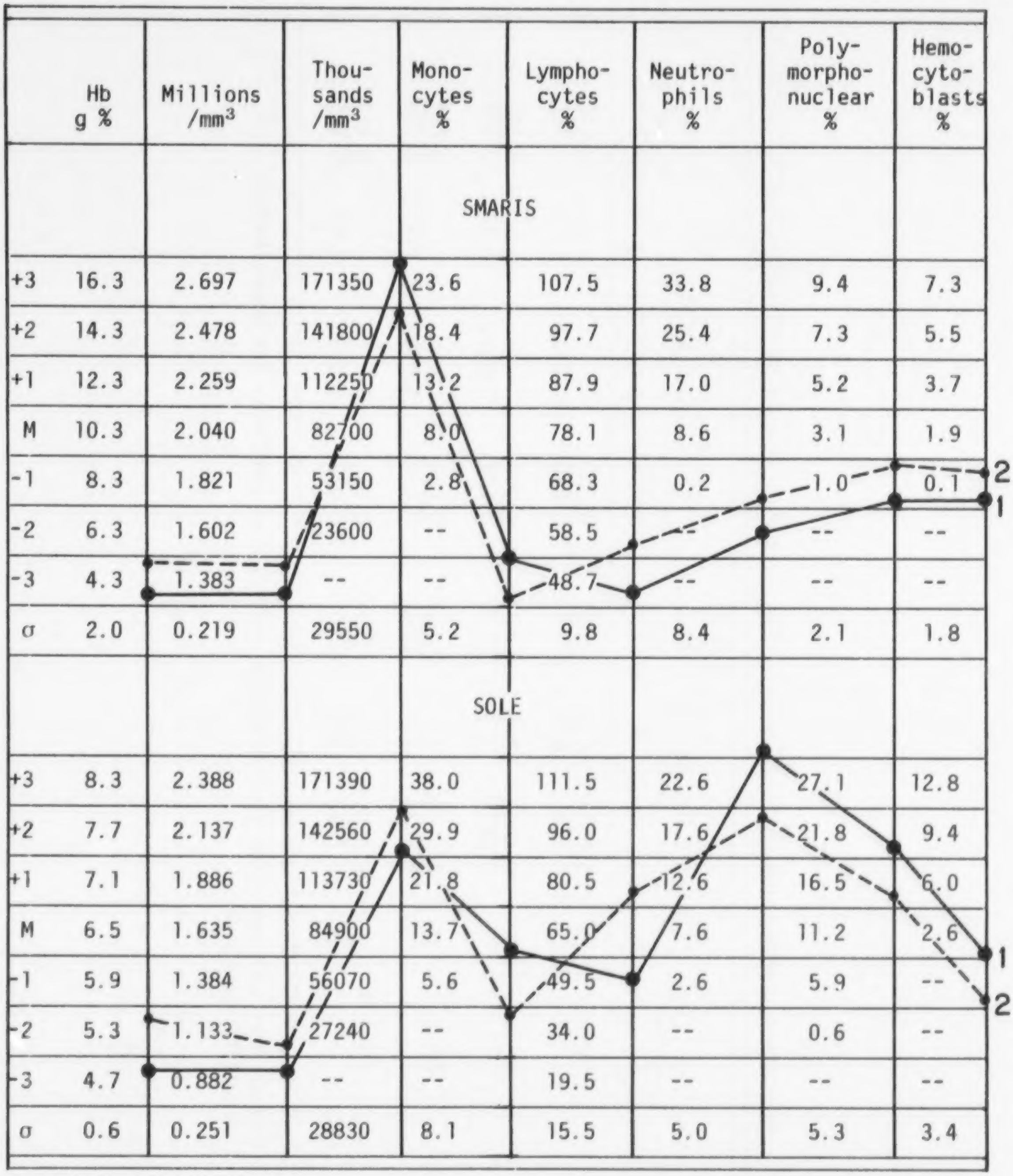
Normoblasts and polychromatophilic erythrocytes were often found in the blood smears. There was a significant number of immature erythrocytes with unevenly stained cytoplasm, indicating irregular distribution of hemoglobin inside the cells. The agglutination property was very pronounced. An increase in the relative number of immature erythrocytes in the blood of smaris in acute poisoning was maximum, and consisted of 455 cells per 100 fields of vision. The figure was 89 cells with a 1.0 mg/liter concentration; 47 with a 0.5 mg/liter concentration; and 26 cells with a 0.02 mg/liter concentration. The average number of cells in the control fish was 24. In addition to the above changes, signs of primitive cell-hemocytoblasts (up to 2.6%) were also observed in the blood smears. Vacuolization of the cytoplasm was observed in the white blood cells. The changes observed in the qualitative composition of the red and white blood in marine fish in petroleum poisoning were nonspecific.

TABLE 2. FRACTIONAL COMPOSITION OF BLOOD HEMOGLOBIN IN FISH IN POISONING BY DISSOLVED PETROLEUM PRODUCTS

Type of Experiment	Duration of Experiment	N	C Met Hb		C Total Hb -SHb	C HbO_2	C SHb		Total Pigment	Total Inactive Pigment
			mEq/liter	%	mEq/liter	mEq/liter	mEq/liter	%	mEq/liter	%
Acute	10 h	10	0.0010	2.0	0.051	0.050	---		0.051	2.0
Smaris		10	0.0040	6.0	0.063	0.059	0.0030	4.5	0.066	10.5
Acute	24 h	10	0.0003	0.9	0.032	0.031	---		0.032	0.9
Sole		10	0.0010	4.2	0.023	0.022	0.0010	4.2	0.024	8.4
Subacute	24 h	10	0.0010	1.5	0.066	0.065	---		0.066	1.5
		10	0.0030	5.3	0.055	0.052	0.0008	1.4	0.056	6.7
Smaris	72 h	10	0.0005	1.3	0.039	0.038	---		0.039	1.3
		10	0.0020	3.4	0.059	0.052	0.0005	0.8	0.059	4.2

Note: The numerator indicates the controls; the denominator indicates the experimental fish; n = number of specimens examined.

TABLE 3. HEMATOLOGICAL INDICES IN ACUTE AND SUBACUTE PETROLEUM POISONING IN FISH



	Hb g %	Millions /mm^3	Thousands /mm^3	Monocytes %	Lymphocytes %	Neutrophils %	Polymorphonuclear %	Hemocytoblasts %
				SMARIS				
+3	16.3	2.697	171350	23.6	107.5	33.8	9.4	7.3
+2	14.3	2.478	141800	18.4	97.7	25.4	7.3	5.5
+1	12.3	2.259	112250	13.2	87.9	17.0	5.2	3.7
M	10.3	2.040	82700	8.0	78.1	8.6	3.1	1.9
-1	8.3	1.821	53150	2.8	68.3	0.2	1.0	0.1
-2	6.3	1.602	23600	--	58.5	--	--	--
-3	4.3	1.383	--	--	48.7	--	--	--
σ	2.0	0.219	29550	5.2	9.8	8.4	2.1	1.8
				SOLE				
+3	8.3	2.388	171390	38.0	111.5	22.6	27.1	12.8
+2	7.7	2.137	142560	29.9	96.0	17.6	21.8	9.4
+1	7.1	1.886	113730	21.8	80.5	12.6	16.5	6.0
M	6.5	1.635	84900	13.7	65.0	7.6	11.2	2.6
-1	5.9	1.384	56070	5.6	49.5	2.6	5.9	--
-2	5.3	1.133	27240	--	34.0	--	0.6	--
-3	4.7	0.882	--	--	19.5	--	--	--
σ	0.6	0.251	28830	8.1	15.5	5.0	5.3	3.4

Note: 1 - 25.0 mg/liter concentration of petroleum; time 5-7 hours;
2 - 19.0 mg/liter; time 3 days.

The disturbances in carbohydrate metabolism in the first phase of poisoning were manifested by pronounced hyperglycemia, an increase in the amount of glycogen, and a decrease in the lactic acid in the blood (Table 4).

TABLE 4. CHANGES IN CARBOHYDRATE METABOLISM INDICES IN THE BLOOD OF SMARIS AND SOLE IN ACUTE POISONING BY DISSOLVED PETROLEUM PRODUCTS

Time, Hours	No. of Fish Examined	Glucose, mg% M ± m	Glycogen, mg% M ± m	Lactate, mg% M ± m
		SMARIS		
Starting Point	8	55.0 ± 6.0	35.8 ± 2.8	76.7 ± 8.9
2	8	71.6 ± 7.4	47.4 ± 4.2	43.6 ± 7.1
4	8	109.7 ± 5.6	55.9 ± 1.1	30.2 ± 5.9
6	8	79.2 ± 4.4	54.2 ± 2.2	36.2 ± 3.1
8	8	49.3 ± 5.2	51.1 ± 3.4	48.7 ± 3.6
10	8	26.2 ± 4.8	48.2 ± 1.9	60.6 ±10.0
Controls	8	61.2 ± 4.2	29.4 ± 3.5	73.5 ± 7.3
Time, Hours		SOLE		
Starting Point	12	30.2 ± 3.3	33.5 ± 4.4	25.0 ± 3.9
1	14	41.0 ± 1.1	61.9 ± 3.7	16.1 ± 3.6
2	12	54.8 ± 7.7	53.5 ± 3.5	17.4 ± 2.6
3	13	24.4 ± 2.4	50.5 ± 5.0	28.0 ± 1.5
Controls	14	35.1 ± 1.7	43.7 ± 3.6	39.5 ± 2.7

The significant hyperglycemia in the first half of the experiment indicated intensive glucose transport to organs and tissues, primarily to the central nervous system, as the most important energetic substrate. The simultaneous hypolactacidemia was probably the result of decreased glycolysis. Accumulation of glycogen in whole blood cells apparently promotes its synthesis from plasma glucose by white blood cells. The second part of the experiment was characterized by pronounced hypoglycemia and a decrease in the concentration of glycogen in the blood of the poisoned fish. In this case, the organism responds to the effect of the toxic factor by progressive deterioration in its condition on all levels.

The chronic experiments were conducted with a wide range of concentrations of dissolved petroleum products; the 0.01 mg/liter dose caused no significant changes in the carbohydrate metabolism indices in the blood of smaris. Carbohydrate metabolism in the tissues of the fish also changed significantly in poisoning from dissolved petroleum hydrocarbons. There was a progressive decrease in carbohydrate reserves in all tissues studied in acute poisoning in smaris (Table 5).

TABLE 5. CONCENTRATION OF GLYCOGEN (M ± m mg%) IN SMARIS TISSUES IN ACUTE POISONING BY DISSOLVED PETROLEUM PRODUCTS

Hours	N	Glycemia mg %	Liver	Heart	Red Muscles	Brain
Controls	14	59.9 ± 6.3	625 ± 73	440 ± 39	251 ± 41	35 ± 1
2	14	*71.3 ± 8.6	*390 ± 63	347 ± 35	170 ± 23	36 ±11
4	14	*82.4 ± 1.2	*380 ± 60	*168 ± 25	*153 ± 19	20 ± 3
10	15	---	*365 ± 64	*261 ± 47	*130 ± 26	---

Note: * indicates significant results $p < 0.05$.

The degree of these changes in the different tissues was almost identical: approximately 50% of the control values, i.e., the disorders in glycogen metabolism in the liver, heart and red muscles and brain were similar in nature. The changes in the concentration of polysaccharides in the different tissues were similar - the fundamental disorders in glycogen metabolism began in the first hours of contact with the toxic substances and gradually increased in time. When sections of intoxicated liver were incubated in saline solution, a slight decrease was observed in production of glucose in comparison with intact sections. Acute poisoning also caused a decrease in glycogen in the liver and production of glucose by sections of this organ in sole, and the latter process was very pronounced.

The decrease observed in the concentration of glycogen in the livers of the poisoned fish, accompanied by a decrease in glucose production by the sections, was apparently due to processes for detoxifying petroleum hydrocarbons which principally occur in the liver. The glucose formed from the glycogen is used as a source of energy for detoxification reactions and also as a substrate for metabolic conversions of the petroleum products.

Subacute poisoning of smaris and sole resulted in different disorders in glycogen metabolism in the various tissues. A hepatotoxic effect was found in smaris - a more significant decrease in glycogen in the liver in comparison to the other tissues. Glycogen metabolism in heart muscle was primarily and more significantly disturbed in sole, and inhibition of hepatic glycogen function was observed only after seven days of exposure. Under the conditions of this experiment, sections of liver from both species of fish exhibited a significant decrease in glucose production, but cardiac muscle from the sole instead exhibited increased consumption of sugar from the incubation medium in comparison to the controls.

The experiments with the highest concentration which caused chronic toxicosis in smaris (9 mg/liter) were conducted in different seasons of the year: spring-summer, which corresponded to the pre-spawning and beginning of the spawning periods, and in the autumn, when smaris are in the post-spawning period. In these conditions, the hepatotoxic effect of the petroleum products was primarily manifested (Table 6).

TABLE 6. CONCENTRATION OF GLYCOGEN (M±m, mg%) IN SMARIS TISSUES IN CHRONIC POISONING BY DISSOLVED PETROLEUM PRODUCTS (9 mg/liter)

Exposure Time (days)	Liver	Heart	Red Muscle	White Muscle
SPRING-SUMMER				
7	508 ± 15 (8) *292 ± 35 (19)	297 ± 39 267 ± 24	149 ± 13 140 ± 13	55 ± 8 71 ± 4
15	242 ± 71 (11) 176 ± 29 (17)	232 ± 17 247 ± 20	93 ± 15 116 ± 12	41 ± 3 *60 ± 7
20	242 ± 71 (11) 183 ± 36 (10)	232 ± 17 248 ± 37	93 ± 15 69 ± 6	41 ± 3 61 ± 7
25	163 ± 38 (6) *41 ± 11 (8)	74 ± 23 33 ± 7	38 ± 8 43 ±10	35 ± 5 *60 ± 3
AUTUMN				
5	392 ± 87 (6) *115 ± 26 (9)	286 ± 33 252 ± 28	79 ± 5 91 ± 18	60 ± 7 *35 ± 5
15	427 ± 84 (6) *161 ± 15 (6)	319 ± 31 386 ± 54	80 ± 11 *123 ± 11	90 ± 6 80 ± 5
20	427 ± 84 (6) *174 ± 14 (6)	319 ± 31 366 ± 61	80 ± 11 130 ± 14	90 ± 6 67 ±12
25	488 ± 84 (6) *156 ± 36 (6)	359 ± 13 *470 ± 45	70 ± 11 92 ± 17	51 ± 8 52 ± 3

Note: The number of specimens examined is indicated in parentheses;
* Indicates the significance of the results $p<0.05$.

In both variants (spring and autumn), the greatest decrease in the concentration of glycogen was observed after 5-7 days of exposure. However, the decrease in the polysaccharide reserves in the liver of the poisoned fish remained at approximately the same level in the autumn experiments--30-38% of the control values--during the entire experiment, while the polysaccharide reserves in the poisoned fish did not significantly differ from the control values on the 15th and 20th days in the spring variant. A pronounced decrease in liver glycogen reserves in the poisoned fish was only observed at the end of the experiment, on the 25th day.

The significant decrease in the concentration of glycogen in the liver in the initial period of both variants of the experiment was apparently due to processes for detoxication of the dissolved petroleum products, since it has been shown that microsomal enzymes in the liver of fish actively metabolize various petroleum products, included in the detoxication process as a basic component of glucose. However, the metabolic reaction of the fish in the spring and autumn experiments differed; this was apparently due to the different physiological condition of the animals. In the pre-spawning period, all metabolic processes were highly active, indicating a high degree of induction activity of microsomal enzymes which participate in detoxication of heterologous compounds and their excretion from the organism. This allows for more rapid metabolization of the petroleum products and for the active elimination of the metabolites. The results of this toxic effect of the dissolved petroleum products decreased, and a state of equilibrium was observed in glycogen metabolism at some time (data on the 15th and 20th days). We can thus conclude that the liver of smaris is more resistant to the effect of petroleum products in the pre-spawning period than in the post-spawning period. As the concentration of dissolved petroleum products used in the experiment decreased, the effect of compensatory mechanisms appeared: The decrease in the concentration of glycogen in the liver was followed by restoration of glycogen reserves and then another decrease. As a result, the evolution of the toxic process was triphasic in nature. The changes in the concentration of glycogen in the tissues of smaris could be considered fluctuations in the normal level only with the 0.01 mg/liter concentration. Liver sections from fish exposed to this concentration for 30 days precipitated glucose in amounts which did not differ from those of the controls.

Determination of several enzymes involved in carbohydrate metabolism in the tissues of the fish showed that the changes observed in the concentration of glycogen were first related to a decrease in the glycogen-synthesizing enzyme in the liver and second, to an increase in the activity of glycogen-splitting enzymes such as glycogenphosphorylase and -amylase. The change in the activity of glycogenolytic enzymes did not always occur concomitantly. The decrease in the glycogen reserves in the livers of the poisoned fish was also caused by an increase in glucose-6-phosphatase activity. The activity of this enzyme in the brain changed in the same way as in the liver. However, the high glucose-6-phosphatase activity in the brain corresponded with a high concentration of glycogen in the brains of the sole in comparison with the smaris. Glucose split from glucose-6-phosphate apparently plays a substantial role in cerebral metabolism in sole in a toxic environment; cerebral metabolism thus becomes more independent of the level of glycemia, which is an additional factor determining the high resistance of this species to toxic substances.

The studies which we conducted thus indicate that fish can be widely used as objects for monitoring pollution of the marine environment based on a number of their features.

REFERENCES

Balayev, L. A., N. D. Mazmanidi, and R. R. Bazhashvili. 1976. Reactions of some Black Sea hydrobionts in an electrical current field in poisoning by dissolved petroleum products. Vopr. ikhtiologii 16(4).

Fedorov, V. D. 1976. Problem of admissible effects of the anthropogenic factor from an ecologist's point of view. Proceedings of the III VGBO Conference, 1.

Korolev, A. M., Yu. Yu. Frolov, and N. D. Mazmanidi. 1978. Study of the physical-chemical properties of mucus from Black Sea smaris in conditions of petroleum toxicosis. Biol. nauki, 9.

Mazmanidi, N. D. 1974. Symptoms of petroleum poisoning in hydrobionts. Rybnoye khoz-vo, 9.

Mazmanidi, N. D. 1977. Effect of petroleum poisoning on oxygen saturation of the blood of the Black Sea fish. Rybnoye khoz-vo, 6.

Mazmanidi, N. D. et al. 1972. Study of the effect of petroleum pollution of seas on hydrobionts. Scientific report on subject No. 21, VNIRO, Moscow.

Mazmanidi, N. D., and L. A. Balayev. 1974. Effect of petroleum poisoning on the behavior of some Black Sea fish in an electrical current field. Rybnoye knoz-vo, 11.

Mazmanidi, N. D., and G. I. Kovaleva. 1972. Experimental data on the effect of petroleum on some chemical properties of the marine environment. Okeanologiya, 5.

Mazmanidi, N. D., and G. I. Kovaleva. 1975. Effect of dissolved petroleum products on some elements in carbohydrate metabolism in fish and invertebrates. Vopr. ikhtiologii, 15(5).

Mazmanidi, N. D., G. I. Kovaleva, and N. A. Zobova. 1975. Determination of petroleum products and naphthenic acids dissolved in sea water. Okeanologiya, 15(3).

Mazmanidi, N. D., and N. P. Zambakhidze. 1974. On pathological-anatomical changes in fish in acute and subacute poisoning by petroleum. Trudy Gruzinskogo otd. VNIRO, 16.

Rice, S. D., J. V. Short, and J. F. Karinen. 1976. Effects of hydrocarbons on biological systems: behavioral, physiological and morphological; toxicity of Cook Inlet crude oil to several Alaskan marine fishes and invertebrates. Symposium on sources, effects and sinks on hydrocarbons in the aquatic environment, Washington D.C., U.S.A.

Stroganov, N. S. 1976. Comparative sensitivity of hydrobionts to toxic substances. General ecology. Biocoenology. Hydrobiology, 3 (Scientific and technical results. VNITI AN SSSR), Moscow.

IMPROVED ASSESSMENT OF ECOLOGICAL EFFECTS BY INCORPORATING PHYSICAL SIMULATIONS IN BIOASSAY PROCEDURES

by

D. J. Baumgartner
Marine Division, Environmental Research Laboratory
U.S. Environmental Protection Agency
Corvallis, Oregon

INTRODUCTION

Bioassay procedures are customarily used in the United States by marine pollution control regulatory authorities to determine the acceptability of municipal and industrial wastes for disposal in the ocean. In some cases the results of bioassays are of advisory value while in other cases, such as disposal of material dredged from waterways, failure to pass one or more bioassay tests can result in denial of a permit. Since a permit is required for ocean dumping from barges and for continuous operation of an ocean outfall, denial of a permit can result in significant economic and technical difficulties for municipalities, industries, and dredging authorities. Consequently, dischargers as they may be conveniently labeled, are urging regulatory authorities to adopt bioassays that are realistic indicators of environmental stress, but which are not arbitrarily restrictive.

Pollution control authorities are, of course, also interested in reliable indicator tests, and since they are responsible for protecting the environment they are motivated to prescribe tests that will be effective in screening out chemicals and wastes that are likely to be troublesome. Recognizing that laboratory bioassays are neither infallible, nor exact representations of the environment, authorities are quite conservative in adopting new techniques that are projected as being more realistic. It seems to this observer that pollution control authorities, at least in the United States, favor standards and uniform tests justified no doubt on legal grounds as well as administrative ease, whereas many scientists generally tend to favor tests that are tuned to the conditions of the specific case, rather than being standardized.

In recent years major advances in bioassay testing have been incorporated in the administration of waste discharge permits, because of changes in the legal basis for pollution control and the tremendous economic impact of permit decisions. There are however improvements which are still necessary and the purpose of this paper will be to describe a few that are motivated by consideration of the physical and chemical factors influencing the distribution of wastes discharged from barges and pipelines.

PRESENT BIOASSAYS

Both the Environmental Protection Agency and the Corps of Engineers have published bioassay manuals for evaluation of ocean dumping (USEPA 1978; U.S. Army Engineers 1977). Generally mortality is the endpoint of the bioassay even though it is recognized that ecological significance is difficult to interpret directly from this information. The only nonlethal tests being utilized are the phytoplankton growth tests and one attempting to evaluate bioaccumulation of toxic chemicals, again recognizing that interpretation is difficult. Federal ocean dumping permit regulations require use of bioassays for the liquid phase, suspended phase, and sediment phase.

Most tests are conducted in aquaria without flowing seawater (sometimes called "static" bioassays) although the recommended procedure for the solid phase bioassays employs flow-through aquaria. In this test, the benthic animal survival is determined after 10 days, under a 15-mm blanket of material to be tested, but in other tests the LD_{50} is calculated for exposures of 4 to 96 hours, using at least three concentrations of test solution. Results are compared to the expected concentrations to be found in the dumping zone using field data or model results. For ocean outfall permits a standard test of bioassay requirements does not yet exist.

INCORPORATION OF TIME VARYING FACTORS IN BIOASSAYS

Ocean Outfalls

Many models in use today have been developed from the same simple plume mechanics that have been found to adequately describe the behavior of smoke plumes. In Figure 1 the main features of an ocean outfall plume are sketched. The features which are important in relationship to the regulatory use of bioassays are: (a) the rising plume; (b) the transition zone; and (c) the drift flow. These three zones are shown again in Figure 2 in terms of the relative concentrations and length of times to which organisms are exposed. The initial dilution rates are based on plume calculations for typical ocean outfall configurations (Baumgartner 1971; Teeter and Baumgartner 1979). The example used in Figure 1 showing an initial dilution of 100 to 1 in one minute is in the range of dilutions frequently found, i.e., between 50/1 and 300/1 within several minutes at most.

In order to operate a bioassay chamber in which the appropriate dilution rate can be achieved in such a way as to expose the assay organisms it would be necessary to compute the expected dilution according to accepted models and adjust the plumbing controls accordingly. The reliability of this initial dilution estimate is in the range of ± 20% (Brooks 1973) and therefore is considerably better than many other environmental predictions. The range of values usually prescribed for bioassays should conveniently bracket this estimate. The bioassay organisms used in this test should be the planktonic and fish species selected for the region. (Benthic organisms would not be expected to be exposed in nature to this concentration/exposure regime). The 96 hour (5,760 minutes) steady state bioassay, if applicable at all, is seen

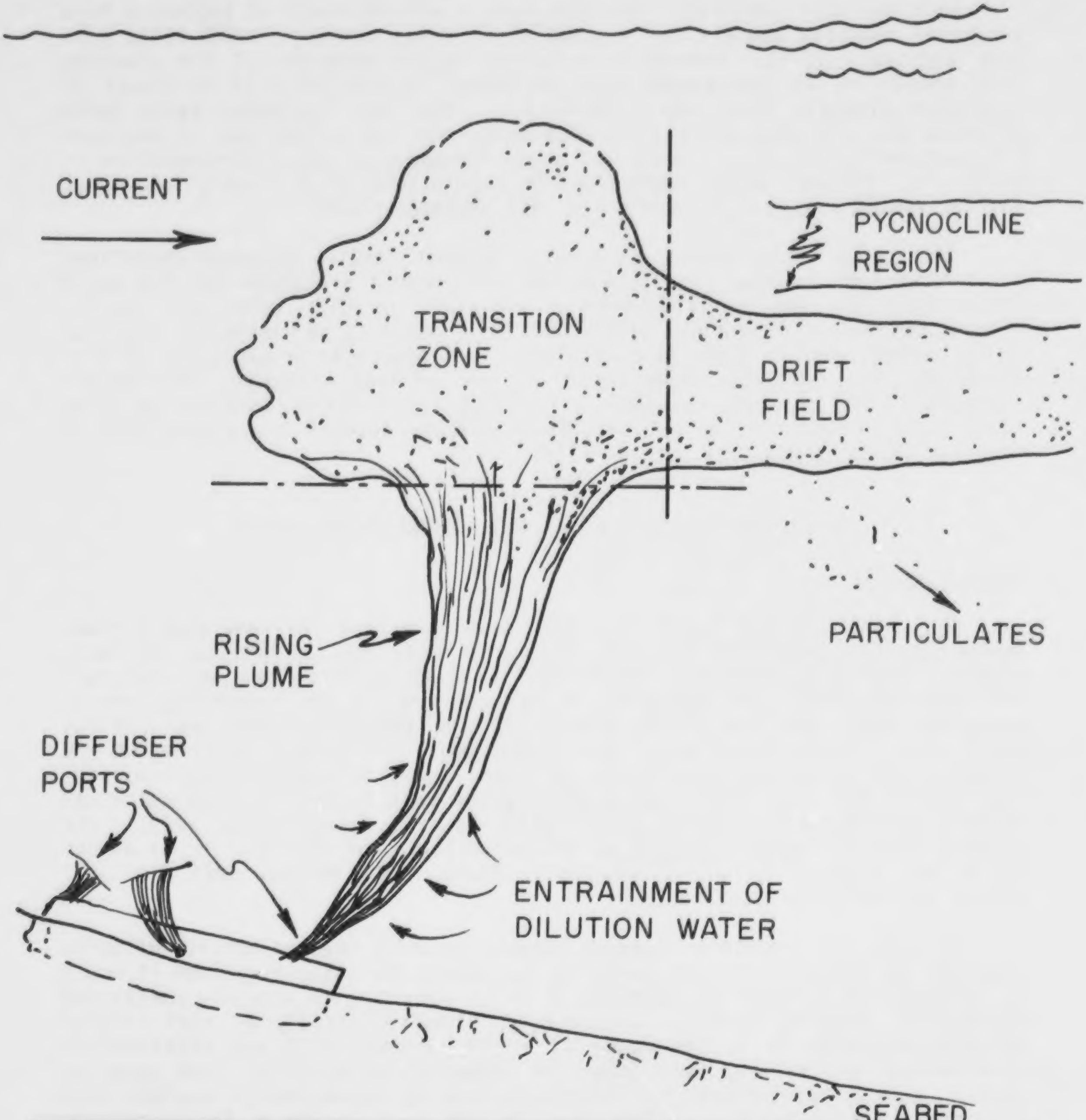

CURRENT
PYCNOCLINE
REGION
TRANSITION
ZONE
DRIFT
FIELD
PARTICULATES
RISING
PLUME
DIFFUSER
PORTS
ENTRAINMENT OF
DILUTION WATER
SEABED

Figure 1. Waste field generated by simple ocean outfall.

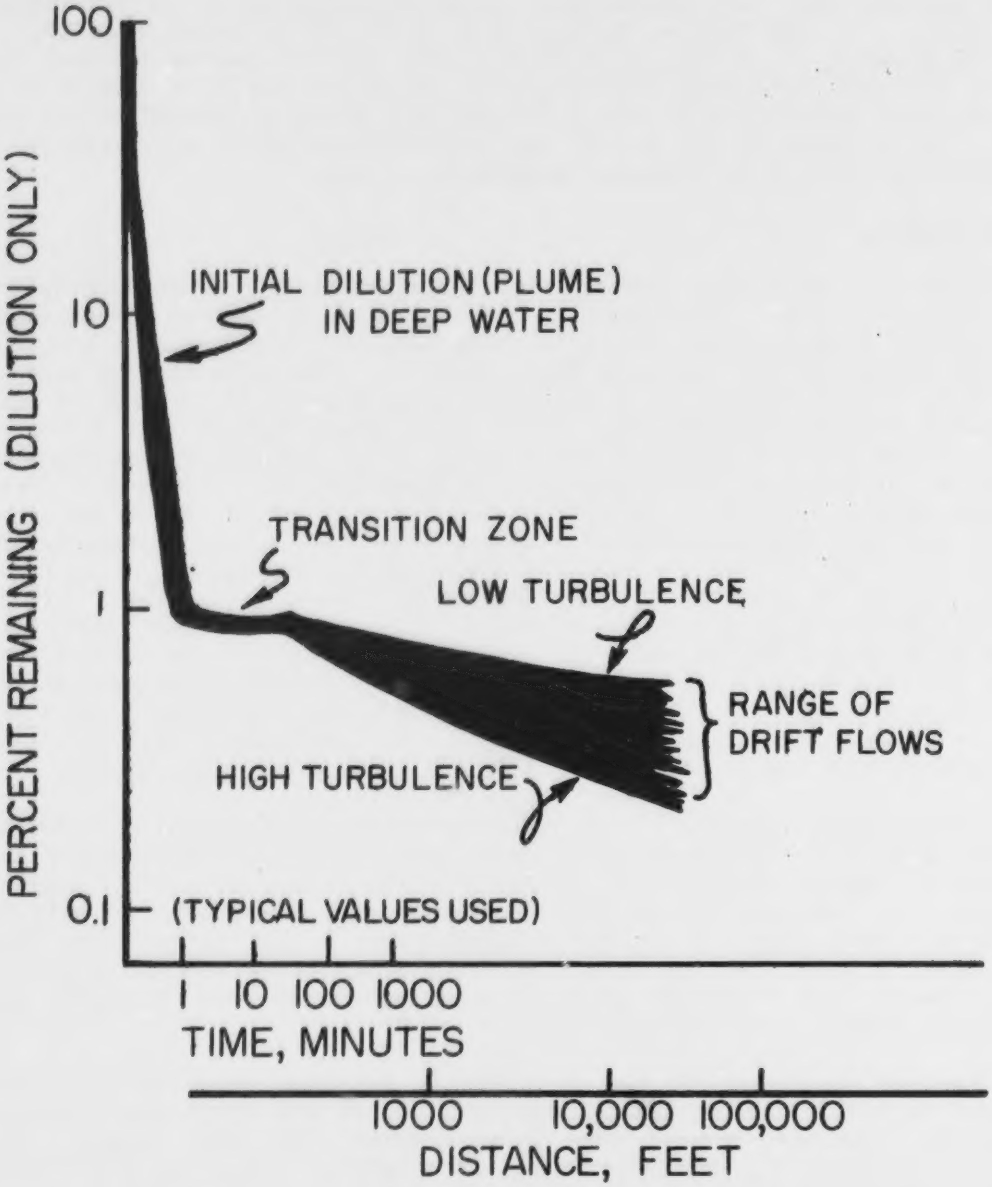


Figure 2. Relative efficiency of dilution in flow regimes associated with municipal outfalls.

to be applicable only to the drift flow region, and only for concentrations that result after initial dilution has been achieved in the rising plume. Even in this case the test would be more appropriate if concentrations were decreased in time, at a rate found in field observations or as predicted by calculations of expected dilutions and pollutant decay. It may be possible to incorporate these changes in concentration in the drift flow region into bioassays so that they would more reasonably represent the environmental conditions. It may however be much more difficult to incorporate the rapid initial dilution phase into the bioassay test, but this may be the most significant exposure experience for organisms caught in the plume. What is most obvious from this figure is that a 96-hour LD_{50} based on concentrations higher than that achieved at the end of the initial dilution is not realistic from the point of view of environmenal exposures.

Barge Dumping

Figures 3 and 4 show schematically two variations in barge dumping models (Clark *et al*. 1971). The convective descent phase is modeled the same way as the buoyant plume of ocean outfalls. The long term dispersion is modeled the same as the drift flow region of ocean outfalls. The barge dumping models are more complicated because of the necessity to account for a wide variety of discharge methods and materials which are dumped, particulates being especially variable. In the case of a single barge dumping event (Figure 3) a bolus of dense waste is rapidly carried downward until reaching a level of buoyant equilibrium. As an example of the use of graphical solutions prepared by Koh and Fan (1968), the top of Figure 5 shows that for a densimetric Froude number (F') of 10 for the initial barge dumping conditions, a penetration depth (Y_f) of 10 times the barge diameter (b_o) would result for a normalized stratification value (E) of approximately 10^{-2}. In this case, then, if the barge were about 8 meters wide, the penetration depth would be about 80 meters in a few minutes. If the depth of water at the dumping site were shallower than 80 meters the waste cloud would immediately begin to deposit particulates in a relatively compact dome on the seabed. The approximate size of the mound is computed from the bottom half of Figure 5 as four times the width.

In the above example, if the depth is considerably greater than 80 meters at the dump site the waste cloud would begin to spread horizontally and be carried by ambient currents. Since the dilution is approximately proportional to the size increase cubed, the concentration of pollutants at this level would be about 1/64 the initial concentration in the barge. The rate of additional dilution in the long term drift flow, as with ocean outfalls, is much slower, and suspended phase bioassay conditions may be quite easily designed to match these expected physical conditions.

Benthic bioassays may be more easily designed when physical factors suggest bottom encounter because the particulate and water phase concentrations are much more precise than in deep water. In water deeper than the penetration depth the sedimentation of particulates is much more difficult to predict because of uncertainties in long term transport calculations.

Another barge dumping configuration frequently employed is discharge into the wake, as in Figure 4. The theoretical analysis of dispersion is not

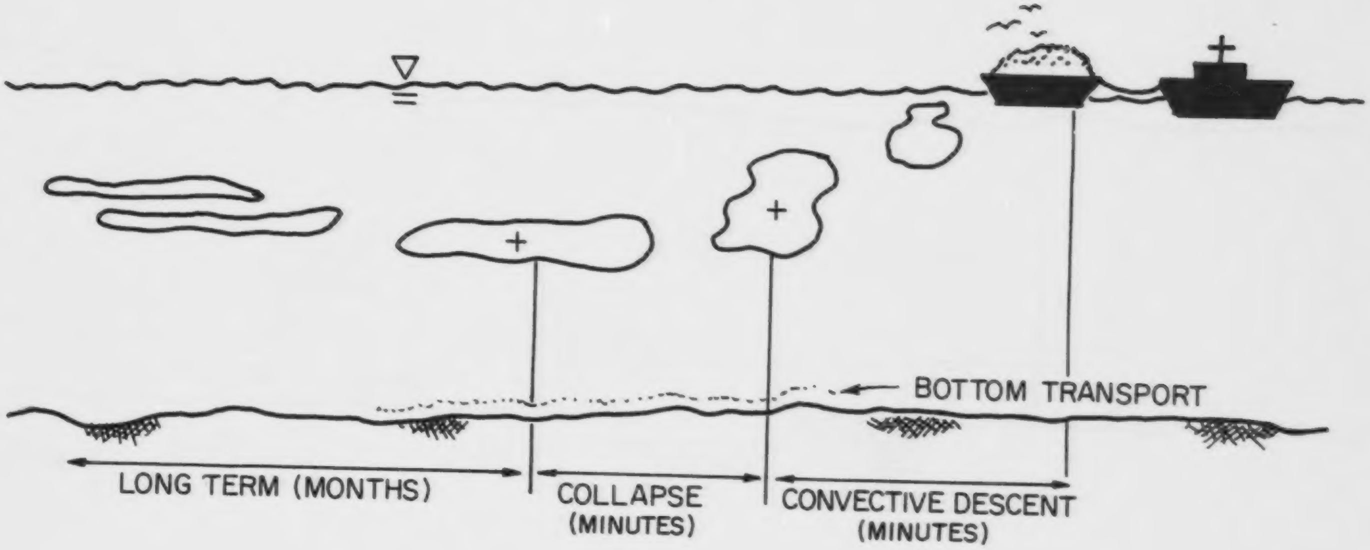

BOTTOM TRANSPORT
LONG TERM (MONTHS)
COLLAPSE
(MINUTES)
CONVECTIVE DESCENT
(MINUTES)

Figure 3. Basic transport phases (after Clark et al. 1971).

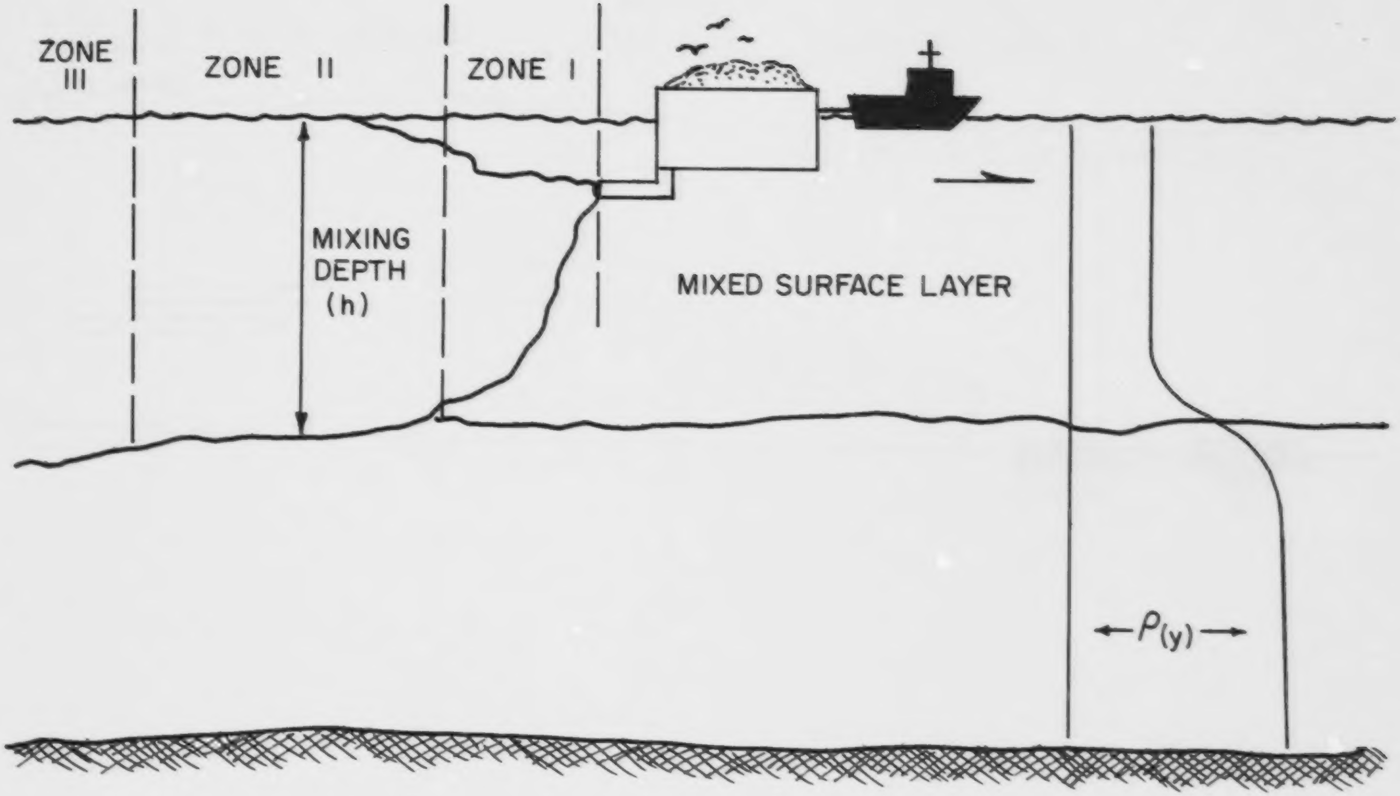
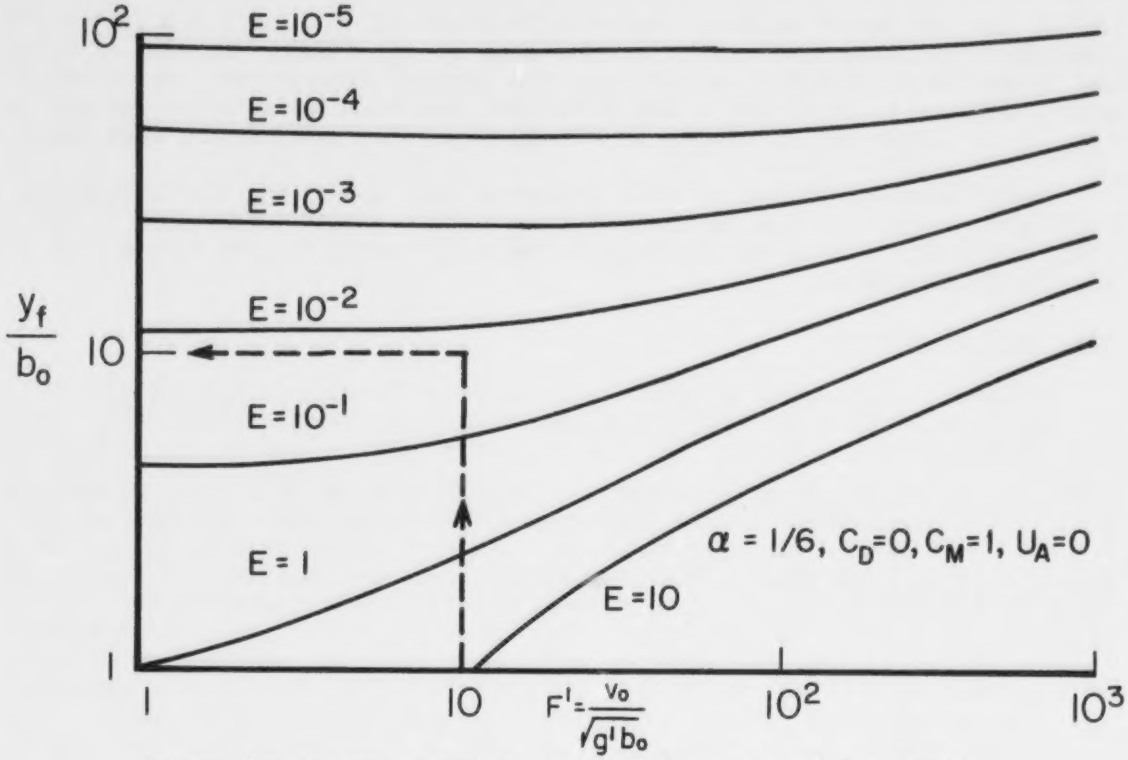

ZONE III
ZONE II
ZONE I
MIXING DEPTH (h)
MIXED SURFACE LAYER
$\rho_{(y)}$

Figure 4. Schematic presentation of fate of material when discharged in barge wake (after Clark et al. 1971).



Convective descent terminal depth (after Koh and Fan 1968).

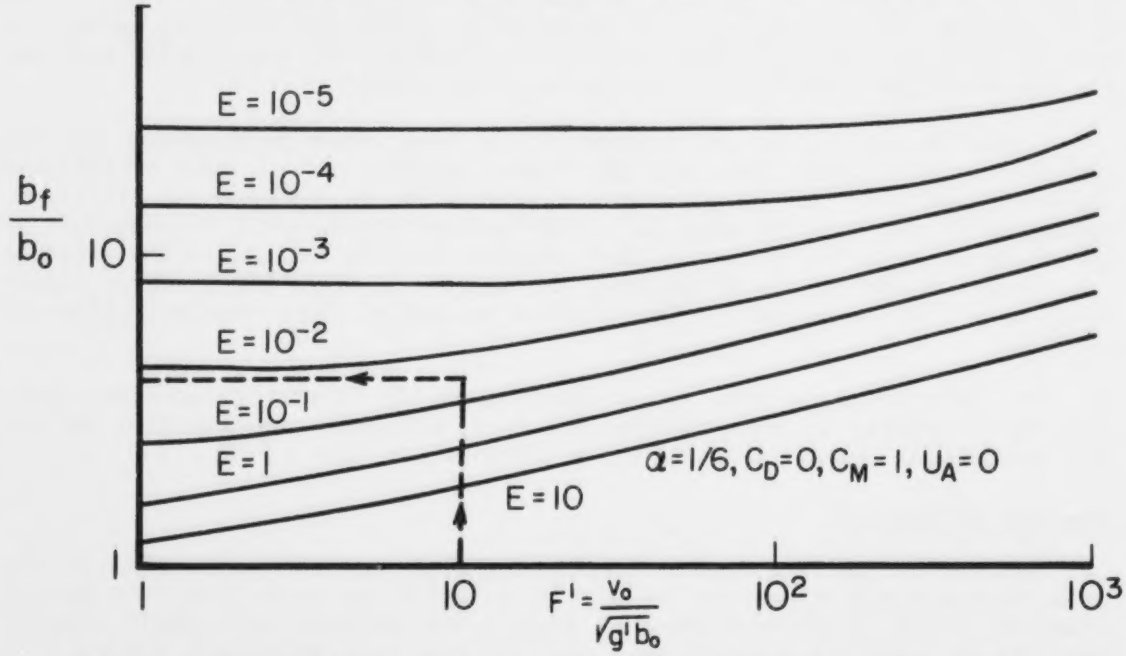


Figure 5. Convective descent terminal size (after Koh and Fan 1968).

unlike jet (or ocean outfall) analysis (Birkhoff et al. 1957) and full scale demonstrations have confirmed this (Abraham et al. 1972). Because of barge and propeller variations as well as the varying arrangements of discharge pipes, especially orifice size and direction, the rates of dilution can easily vary by a factor of 30 (Redfield and Walford 1957; Ketchum and Ford 1952).

Under some variations of wake discharge that appear physically possible, long term dispersion may be described by methods identical to slug releases from barges and continuous discharges from ocean outfalls that form a lens at an intermediate depth.

Long Term Dispersion

Figure 6 graphically displays results of an example (Clark et al. 1971) of long term dispersion according to the method of Koh and Fan (1968). Four situations were considered. In one (U_L) the waste cloud formed a buoyant spherical cloud at 50 meters, and was transported for 8.5 hours before the pollutant concentration at the surface reached a maximum. The surface concentration at that point was between 10^{-4} and 10^{-5} of the initial value. In a second case (C_L) the materials in the waste cloud were described in such a way that the buoyant spherical cloud collapsed into a thin saucer at the initial stage of transport. In this case it was 35 hours before the surface maximum concentration was reached. The concentration would be expected to be about 10^{-6} of the initial concentration. A third case (U_p) describes a spherical mass influenced by a strong pycnocline above the penetration depth. The pycnocline retards vertical migration as seen by the reduced surface concentration of 10^{-7} times the initial value. The fourth case is not described graphically because the surface concentration remained essentially zero. In this example the sea again was considered as possessing a strong pycnocline and in addition the waste material in the cloud was of such nature that the buoyant spherical cloud collapsed into a saucer shape.

These curves, along with other curves that could be computed for the centroid of the waste flow and the seabed surface can be used to describe exposure time and time varying concentrations to be employed in bioassays. Depending on the type of material discharged and the physical arrangements of the barge or outfall, these calculations also provide insight to the relative importance of surface, water column, and seabed in selecting bioassay organisms as well as in evaluating the relative ecological significance of bioassay results.

The above example also shows the possibility of vastly increased variability in results as more complex, but more realistic, environmental factors are considered. Recall that the plume calculations were ± 20%.

Physical Variability

Greater variability occurs with dumping of dredge spoils and sludges than with ocean outfall discharges because the material may range from fine suspensions to large cohesive clumps of clay. The mathematical models used to describe the physical behavior must be quite elaborate to account for the wide range of conditions. This can be accomplished by incorporation of adjustable

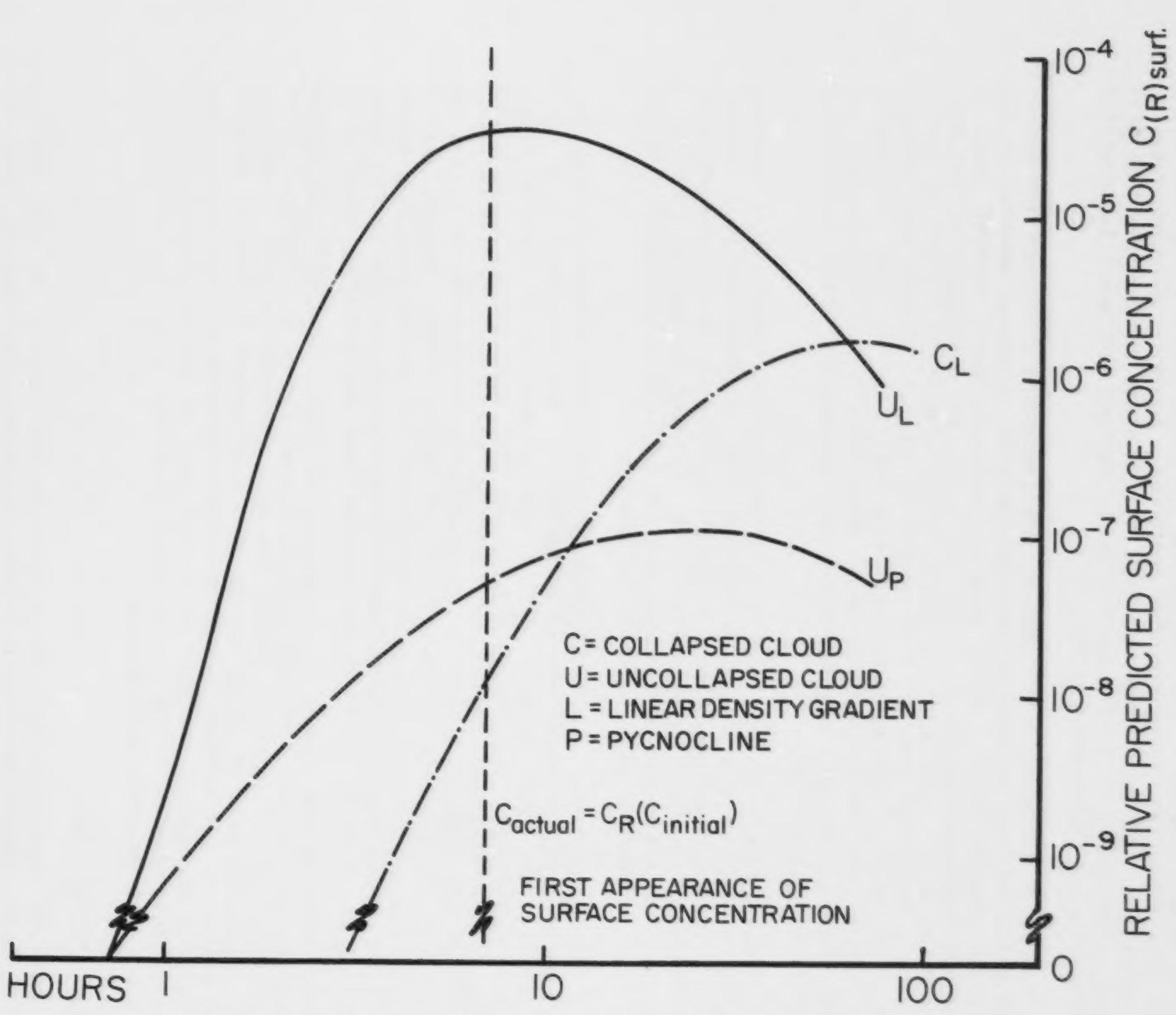


Figure 6. Predicted relative surface concentration for long-term dispersion phase (after Clark et al. 1971).

coefficients. For example, for the illustrations in Figures 5 and 6 a modestly large entrainment coefficient was used as would be appropriate for a liquid or a slurry of nonagglomerated particles. To employ the same entrainment value for a barge of cohesive clays of low water content would be misleading. The unwary or unwise may be led to believe the model is defective rather than a case of misapplication.

Laboratory hydraulic model studies are reasonably efficient for determining values of coefficients to describe behavior of dumped material in the convective descent phase. As an example, hydraulic model studies based on Froude and Reynolds numbers of dredge spoil from the Duwamish River estuary, as conducted at our laboratory suggested an entrainment coefficient of essentially zero for barge dumping in 67 meters of water in Elliott Bay. Using a mathematical model developed by Koh and Chang (1973) we predicted the material would impact the seabed without measurable dilution. Field observations during dumping confirmed the hydraulic and mathematical model predictions (Baumgartner et al. 1978).

Hydraulic models may not be effective for obtaining coefficients relating to barge performance and oceanic turbulence, both of which influence dispersion, chemical interactions, and biological exposure, as do the properties of waste material itself. Consequently it is important to acquire field data, which even in limited circumstances may be sufficient to characterize the coefficients needed to model exposure conditions.

Bioassays may need to be conducted without adequate field information. The range of exposure conditions to be employed in the bioassays, or at least considered before final bioassay design, can be provided by repeated mathematical simulations. Done in an orderly way this exercise may require only one day and can highlight the natural situations which are most influential in controlling the exposure condition. A sensitivity analysis of this sort was conducted on the Koh-Chang model (Teeter and Johnson 1979). Figures 7 and 8 show the range of exposure conditions appropriate for benthic bioassays depending on the values that are determined to be appropriate for two coefficients, α_o and C_{DRAG}. In each figure the top section shows the percent of particulate material reaching the seabed as a function of time, for coefficient values adjacent to each curve. The lower portion of each figure is a measure ($\sqrt{\sigma}$) of the distribution of material around the mass centroid assuming a Gaussian form. These two sets of graphs may be used to determine the mass of material to be placed on a simulated seabed for benthic bioassays. This exercise may well elicit biological insights to be fed back to the physical scientists prior to field studies so that effort might be maximized on those coefficients which are most important biologically.

Chemical Variability

Chemical models can be used to describe the behavior of some chemical species under varying conditions of the environment including salinity, pH, oxygen content (Eh), and the concentration of cations and anions (Ingle et al. 1978). Figure 9 is an example of the effect of salinity on the distribution of silver species as described by the EPA model using data from Jenne et al. (in press). This shows that it may be very important to incorporate time

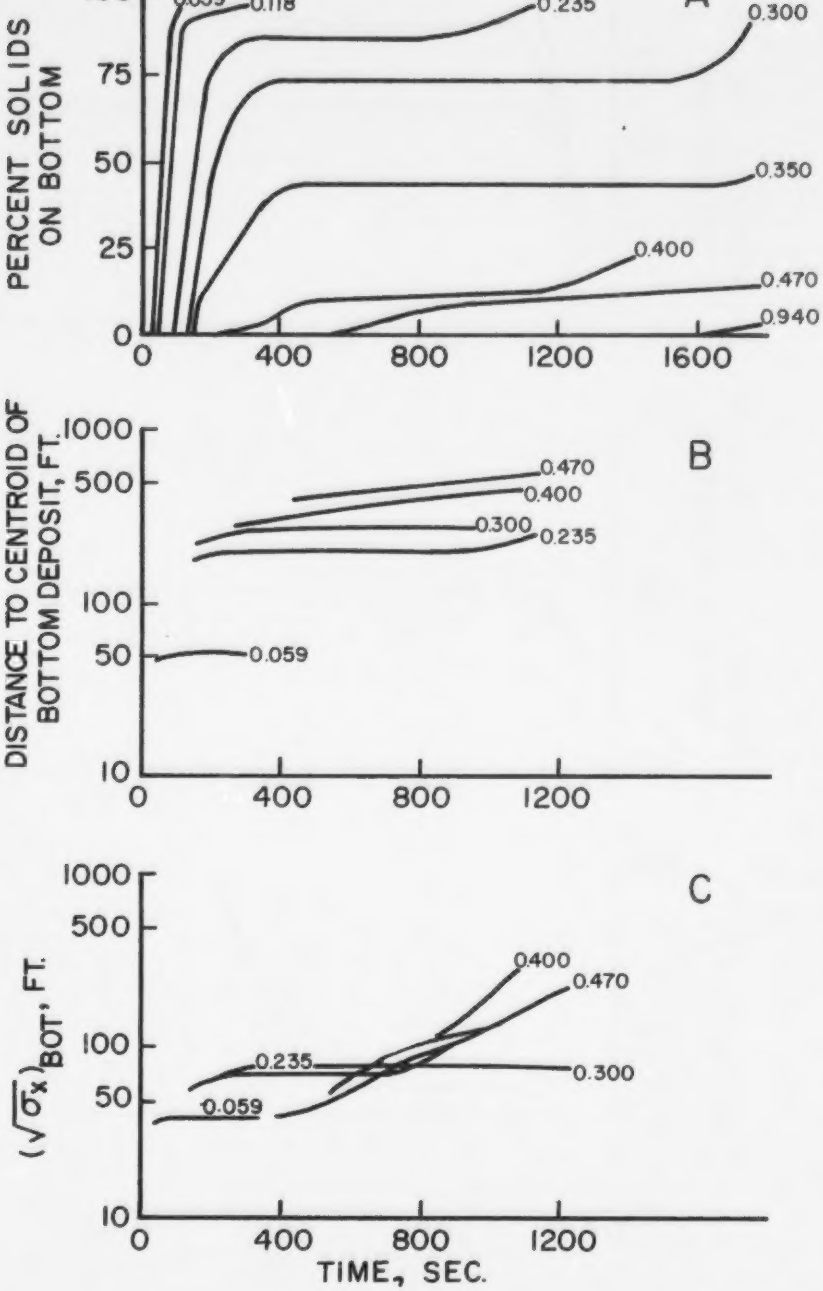


Figure 7. Sensitivity of model output to α_0 in bottom dump disposal (after Teeter and Johnson 1979).

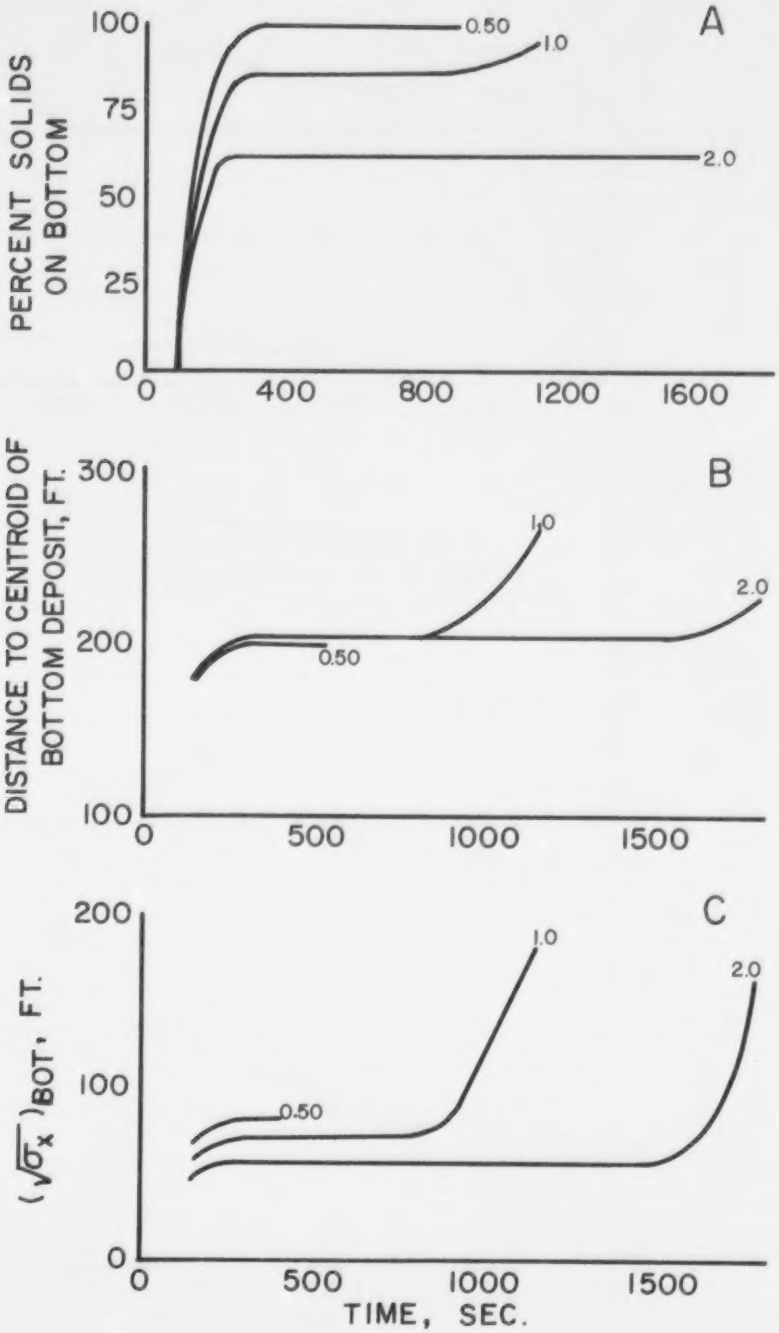


Figure 8. Sensitivity of model output to C_{DRAG} in bottom dump disposal (after Teeter and Johnson 1979).

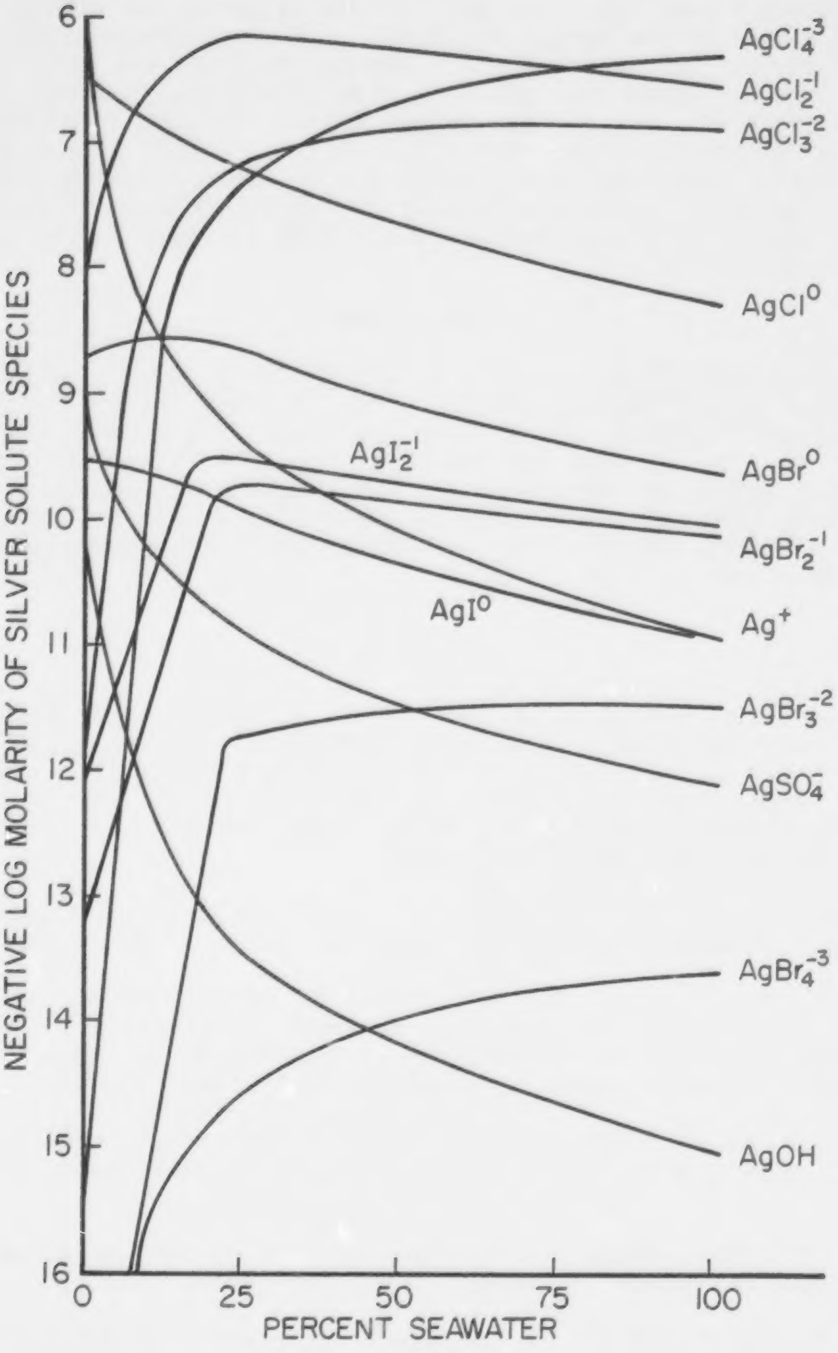


Figure 9. Concentration of silver solute species in freshwater-saltwater mixtures. Total silver concentration: 10^{-6} molar (Ingle and Baumgartner, unpublished).

varying concentrations into bioassays intended to assess the effects of wastes containing silver to the marine environment. As most discharge practices can achieve a mixture 90% seawater within several minutes, the kinetics of such reactions may be important. Assuming that reactions, such as the silver speciation reactions, are as rapid as the physical dilution reactions, or even an order-of-magnitude slower, it is obvious that current bioassays based on 96-hour "static" exposures do not adequately represent exposure conditions in the initial dilution. Figure 9 also demonstrates that exposure conditions in the upper reaches of estuaries (salinity < 25%) are quite different than in the open ocean.

CONCLUSIONS

Recent advances in modeling and in acquisition of data to describe chemical and physical behavior of pollutants have provided the basis for improved bioassay procedures. The benthic bioassays used for the solid phase evaluation of dredge spoils have incorporated some of the advances. Laboratory systems have become more sophisticated and elaborate in response to the need for more effective bioassay procedures and it may now be possible to incorporate time varying concentrations in bioassays through controlled continuous dilution techniques.

REFERENCES

Abraham, G., W. D. Eysink, G. C. van Dam, J. S. Sydow, and K. Miller. 1972. Full scale experiments on disposal of waste fluids into propellor stream of ships. pp. 471-474 In Marine Pollution and Sea Life. Mario Ruivo (ed.) Proc. FAO Tech. Conf. on Marine Pollution and its Effects on Living Resources and Fishing, Rome, Italy, December 19, 1980. Fishing News (Books) Ltd: Surrey and London.

Baumgartner, D. J. 1971. Disposal of liquid and particulate wastes to the ocean. Chemical Engineering Symposium Series. Water 67(107):46-53.

Baumgartner, D. J., D. W. Schults, and J. B. Carkin. 1978. Chemical and physical analyses of water and sediment in relation to disposal of dredged material in Elliott Bay. Army Corps of Engineers Dredged Material Research Program Technical Report D-77-24. 215 pp.

Birkhoff, G., and E. H. Zarantonello. 1957. Jets, wakes and cavities. New York Academic Press. 353 p. (Applied Mathematics and Mechanics, vol. 2).

Brooks, N. H. 1973. Dispersion in hydrologic and coastal environments. U.S. Environmental Protection Agency. EPA-600/3-73-010. 136 pp.

Clark, B. W., W. F. Rittall, D. J. Baumgartner, and K. V. Byram. 1971. The barged ocean disposal of wastes. A review of current practice and methods of evaluation. U.S. Environmental Protection Agency, Corvallis, Oregon. 120 pp.

Ingle, S. E., M. D. Schuldt, and D. W. Schults. 1978. A user's guide for REDEQL.EPA: A computer program for chemical equilibria in aqueous systems. U.S. Environmental Protection Agency, EPA-600/3-78-024, Corvallis, Oregon.

Jenne, E. A., D. C. Birvin, J. W. Ball, and J. M. Burchard. In press. Inorganic speciation of silver in natural waters--fresh to marine, In Environmental Impacts of Nucleating Agents Used in Weather Modification Programs, D. A. Klein, (ed.), Dowder, Hutchinson and Oss, Straudsberg, Pennsylvania.

Ketchum, B. H., and W. L. Ford. 1952. Rate of dispersion in the wake of a barge at sea. Trans. AGU. 33(5):680-684.

Koh, R. C. Y., and Y. C. Chang. 1973. Mathematical model for barged ocean disposal of wastes. U.S. Environmental Protection Agency, EPA-600/2-73-029. Corvallis, Oregon.

Koh, R. C. Y., and L. Fan. 1968. Prediction of the radioactive debris cloud distribution subsequent to a deep underwater nuclear explosion (U). A final report prepared for the Naval Radiological Defense Laboratory, Contract No. N00228-68-C-0684. 266 p.

Redfield, A. C., and L. A. Walford. 1951. A study of the disposal of chemical waste at sea. NAS-NRC Pub. #201.

Teeter, A. M., and D. J. Baumgartner. 1979. Prediction of initial mixing for municipal ocean discharges. U.S. Environmental Protection Agency, Environmental Research Laboratory Report, CERL-043, Corvallis, Oregon. 88 p.

Teeter, A. M., and B. H. Johnson. 1979. A computer study of the Koh-Chang model for dredged material disposal. U.S. Environmenal Protection Agency, EPA-600/3-79-027, Corvallis, Oregon.

U.S. Army Engineer Waterway Experiment Station. 1977. Ecological evaluation of proposed discharge of dredged material into ocean waters. Implementation manual for Section 103 of Public Law 92-532. Vicksburg, Mississippi.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the ocean disposal permit program. EPA-600/9-78-010, Gulf Breeze, Florida.

A PROGRAM FOR INVESTIGATING POLLUTION OF MARINE MACROBENTHOS BY HEAVY METALS AND ITS POSITION IN THE SYSTEM FOR MONITORING THE ENVIRONMENT

by

K. S. Burdin and I. B. Savel'yev
Department of Biology
Moscow State University

Until recently, the collection of information on the state of the environment was typically random. Awareness of the unsatisfactory condition of the environment has occurred after the fact. The global nature of the anthropogenic influence on Earth's biosphere in recent years has led to gradual deterioration of the quality of the human environment. The recently created procedure for assessing the state of the environment on local, regional, national, and global levels has been termed "environmental monitoring." Theoretical and applied aspects of environmental monitoring have been described and discussed in the literature (Gerasimov 1977; Gasilina and Rovinskiy 1977; Simonov 1977). Definitive plans for data flow based on previously developed programs to provide maximum satisfaction of three criteria--observation, evaluation, and prediction of the state of the environment for the purpose of making decisions concerning regulation of the quality of the environment (Izrael' 1977)--are of special interest.

In addition to discussing the generalized schemes for environmental monitoring, biological monitoring and monitoring pollution of the marine biota, concrete programs for diagnostic and prognostic monitoring of heavy metal pollution in marine macrobenthos are discussed, and an attempt to use the proposed programs for monitoring pollution of macrobenthos is presented.

ENVIRONMENTAL MONITORING

Structure of Environmental Monitoring

In this paper, "environment" will refer to the natural and artificial physical and chemical factors and the social changes which could have a direct or indirect effect on the condition of the abiotic and biotic component of the biosphere and on man. With respect to this determination, environmental monitoring should be constructed on the basis of three independent elements: monitoring the natural environment, monitoring the artificial or transformed environment, and monitoring the anthroposphere (Figure 1). "Natural environment" means the natural factors which have not yet been used by man as sources of raw materials or energy, work tools, and consumer goods, but are very

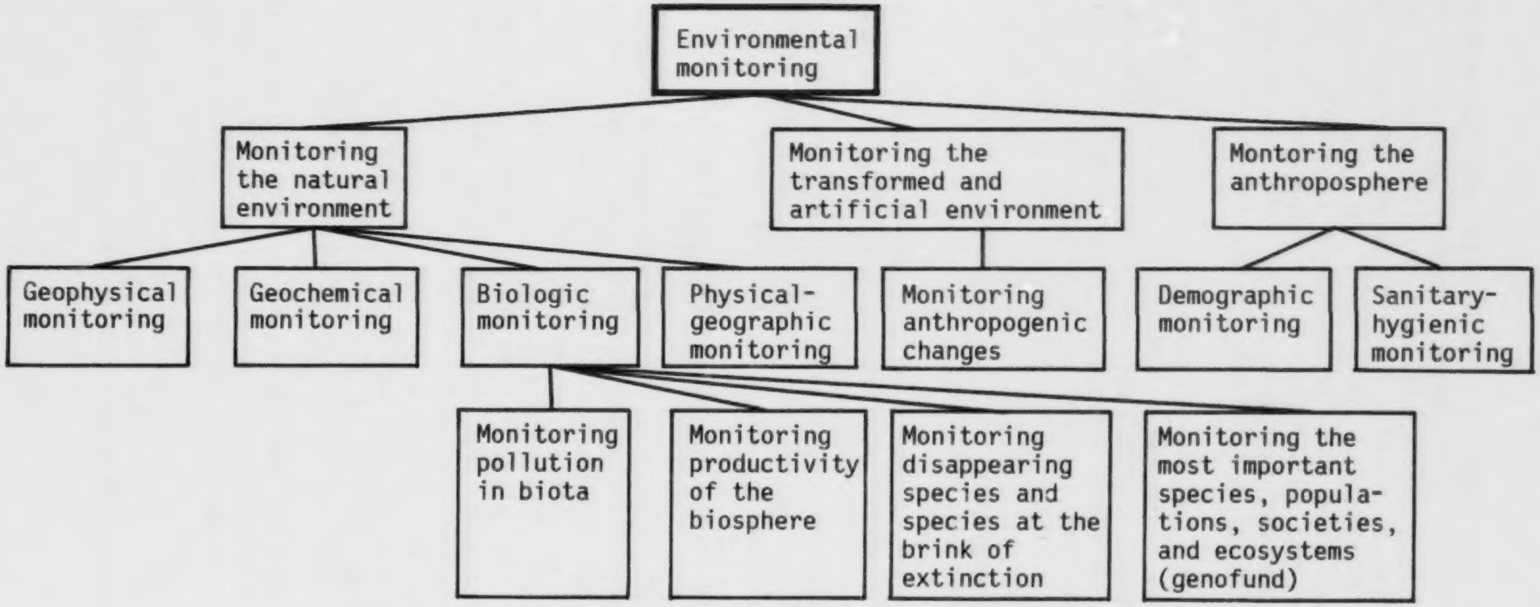

Environmental monitoring
Monitoring the natural environment
Monitoring the transformed and artificial environment
Montoring the anthroposphere
Geophysical monitoring
Geochemical monitoring
Biologic monitoring
Physical-geographic monitoring
Monitoring anthropogenic changes
Demographic monitoring
Sanitary-hygienic monitoring
Monitoring pollution in biota
Monitoring productivity of the biosphere
Monitoring disappearing species and species at the brink of extinction
Monitoring the most important species, populations, societies, and ecosystems (genofund)

Figure 1. Diagram of the environmental monitoring process.

significant for the level of human life and the economic potential of the society. "Artificial environment," most often called the "technosphere" in the literature, includes objects and edifices created by man while working to provide production materials, consumer goods, and the nonindustrial needs of the society. "Transformed environment" chiefly includes the agrosphere used by man to produce food and raw materials from cultivated plants and animals. Figure 1 shows the structure of environmental monitoring which includes monitoring of the anthroposphere, i.e., the population of the Earth, which basically coincides with the technosphere and the agrosphere.

Monitoring the natural environment includes monitoring of the abiotic and biotic components. The basic indices of the condition of the abiotic component of the natural environment, including the lithosphere, the hydrosphere, the pedosphere, and the atmosphere, can be measured within the framework of geophysical, geochemical, and physical-geographic monitoring (Izrael' 1977). Monitoring the anthropogenic changes is the basis for observations, evaluations, and prognosis of the condition of the transformed and artificial environment. Demographic and sanitary-hygienic monitoring can now be used to monitor the anthroposphere.

The condition of the biotic component of the natural environment is observed by biological monitoring, including not less than four types of monitoring at present: monitoring pollution of the biota, monitoring productivity of the biosphere, monitoring disappearing species of animals and plants and species at the brink of extinction, and monitoring the most important species, populations, societies, and ecosystems.

Structure of Monitoring Pollution of the Biota

We know that substances of an anthropogenic origin entering the environment are included in the cycle of matter and consequently appear in the biotic component in some interval of time. Migration and the time in the abiotic component, as indicated above, are observed within the framework of geochemical and geophysical monitoring, while pollution of biota is monitored by observing the level of pollution of the biotic component, movement along the food chain, and the time of accumulation and elimination of pollutants. Monitoring pollution of biota as a function of the living environment is subdivided into monitoring pollution of biota in fresh water, monitoring pollution of biota in salt water, and monitoring pollution of biota on land (Figure 2). Basal monitoring of pollution is now done within the framework of monitoring pollution of biospheric national forests.

We shall examine the structure of monitoring pollution of marine biota which includes monitoring pollution of benthic organisms and monitoring pollution of pelagic organisms. Pollution of benthic organisms is monitored by observing the level of pollution in macrobenthic organisms and meso- and microbenthic organisms (Figure 2). Monitoring pollution in pelagic organisms is based on the trophic relationships of the organisms and can include four types of monitoring--in phytoplankton, zooplankton, fish, and mammals.

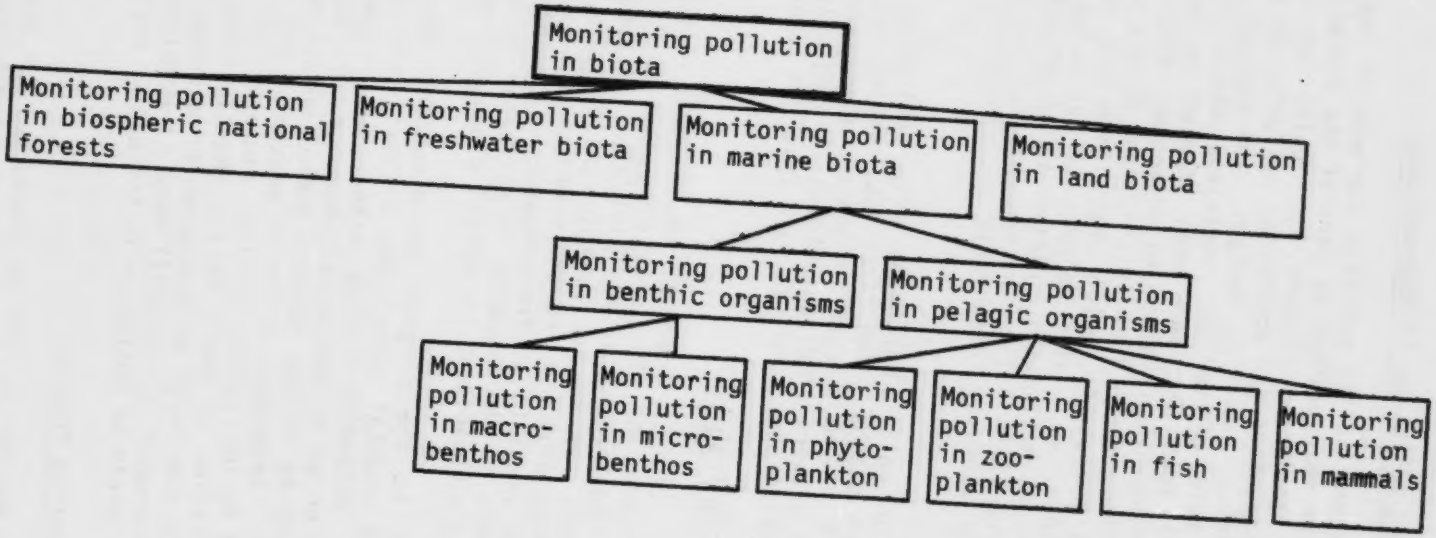

Monitoring pollution in biota
Monitoring pollution in biospheric national forests
Monitoring pollution in freshwater biota
Monitoring pollution in marine biota
Monitoring pollution in land biota
Monitoring pollution in benthic organisms
Monitoring pollution in pelagic organisms
Monitoring pollution in macro-benthos
Monitoring pollution in micro-benthos
Monitoring pollution in phyto-plankton
Monitoring pollution in zoo-plankton
Monitoring pollution in fish
Monitoring pollution in mammals

Figure 2. Diagram of the pollution monitoring process in the biota.

Structure of Monitoring Pollution in Macrobenthos

We shall examine one of the possible variants of the structure of monitoring pollution in the macrobenthos as part of the overall structure of monitoring environmental pollution (Figure 3). Using the spatial and trophic indices to form the structure for monitoring pollution in marine biota, we can distinguish the littoral, neritic, bathyal, and abyssal zones of the ocean. The biogeochemical role of benthic organisms which live in the neritic, bathyal, and abyssal zones has not been studied in depth. Most data on pollution in the macrobenthos relate to organisms which live in the coastal zone. Benthic organisms which live in this zone play a major role in purifying sea water from pollutants which enter from the shore and by way of currents from the open sea. With respect to the existing notions of monitoring and the set of activities related to observation, evaluation, and prognosis, monitoring of pollution in macrobenthos in the coastal zone will require two types of activity--diagnostic and prognostic monitoring of macrobenthos pollution.

The program for monitoring heavy metal pollution of the macrobenthos should include three stages. The first two involve reconnaissance to determine the levels of pollution and prognostic experiments. The third stage involves creating a network of diagnostic monitoring stations whose scale is a function of the problem posed and can vary from the local to the global level.

Diagnostic Monitoring Program

The framework of our approach to diagnostic monitoring is a set of systematic observations of the level of pollution in macrophytobenthos and macrozoobenthos. Measurement of any biological response to any factor of an anthropogenic origin can be included in the diagnostic monitoring program. The capacity of many organisms to store pollutants is one of the most easily detected responses of biota to the presence of pollutants. For this reason, periodic measurement of the quantity of pollution in the living components of the environment is a basic task in current diagnostic monitoring.

With respect to the program for diagnostic monitoring of heavy metal pollution in the coastal zone, the criteria for selecting organisms for monitoring are: extensive living areas, mass, and possible use of the organism as food or as an industrial resource. Biological samples at different geographic points in the world's oceans should be collected at the same time of the year. Potential errors in measuring the concentrations of metals can be minimized by unified collection, storage, and analysis of the samples for the concentration of heavy metals (Martin 1979). A comparison of the available data on the level of pollution of macrobenthos organisms collected at different geographic points will make it possible to detect origins with normal and high levels of pollution in the marine environment.

Prognostic Monitoring Program

Prognostic monitoring can be conducted if data are available on the concentration of heavy metals in sea water and hydrobionts, i.e., a basis for active experiments can be constructed. Any type of experiment on the effect

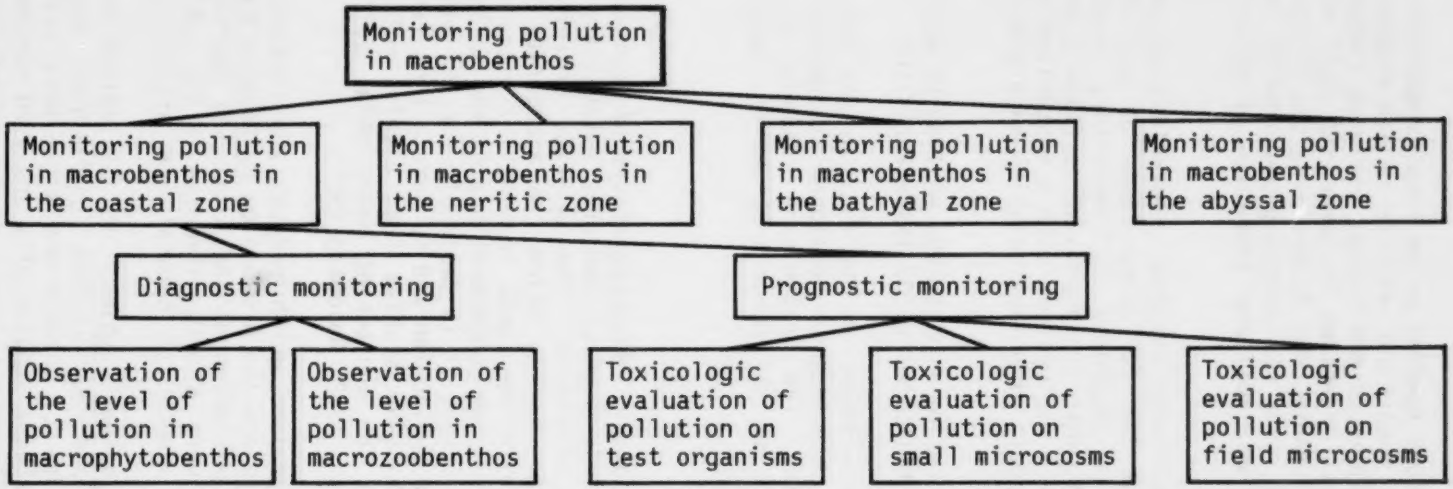

Monitoring pollution in macrobenthos
Monitoring pollution in macrobenthos in the coastal zone
Monitoring pollution in macrobenthos in the neritic zone
Monitoring pollution in macrobenthos in the bathyal zone
Monitoring pollution in macrobenthos in the abyssal zone
Diagnostic monitoring
Prognostic monitoring
Observation of the level of pollution in macrophytobenthos
Observation of the level of pollution in macrozoobenthos
Toxicologic evaluation of pollution on test organisms
Toxicologic evaluation of pollution on small microcosms
Toxicologic evaluation of pollution on field microcosms

Figure 3. Diagram of the pollution monitoring process in the macrobenthos.

of pollutants on individual species or on complex ecological systems conducted in laboratory or field conditions will be called prognostic monitoring. As shown in Figure 3, prognostic monitoring includes conducting laboratory and field experiments on test organisms in large and small microcosms for toxicologic evaluation of pollutants, for determining the limits and rates of accumulation and elimination, and for studying degradation of pollutants in the presence of test organisms used and migration of pollutants along the food chain of the organisms.

Any prognostic monitoring program, regardless of the scale of observation, includes at least the following types of activity: maintenance or cultivation of the test organisms in laboratory conditions, creation of laboratory models of natural ecosystems of different degrees of complexity (static, flowing systems, and systems with a controlled degree of pollution), treating mathematical models of pollution which have predictive value.

A POTENTIAL PROGRAM FOR MONITORING HEAVY METAL POLLUTION IN MACROBENTHOS

We conducted an experiment on the Crimean and Caucasian shores of the Black Sea to implement the program developed to monitor pollution of macrobenthos by heavy metals. The preliminary research primarily consisted of reviewing the literature on the concentrations of copper, zinc, and lead in sea water and hydrobionts.

Diagnostic Monitoring

Selecting the Biomonitor. Representatives of phytobenthos and zoobenthos which have been adequately studied and meet established requirements can be used as the monitoring organisms. The selected organisms should be widely distributed, cosmopolitan species which can accumulate the pollutants under consideration, yet preserve the basic indices for vital activity and genetic stability when relatively high concentrations of the pollutants are present in the environment. In addition, it is desirable for the monitoring organisms to be readily available for collection and to have a long life span. It has been shown in previously published studies that bivalve mollusks and macrophytes are promising organisms for monitoring heavy metal pollution in the marine environment (Berner *et al*. 1972; Goldberg 1975).

Two species of organisms which possess the features listed--the mussel *Mytilus galloprovincialis* and the brown alga *Cystoseira barbata*--were selected to monitor heavy metal pollution of the Black Sea macrobenthos. Colonies of *Mytilus* and *Cystoseira* were almost universal in the sublittoral zone of the Crimean and Caucasian coasts in natural and artificial solid substrates.

Collecting the Biological Samples. The biological samples were collected in the winter of 1977 and 1978 in the sublittoral zone of the Crimean and Caucasian coasts. Samples of mussels and algae were collected at a depth of 0.5-3.0 m; the organisms were subsequently analyzed morphometrically to determine their age. Each dimensional group contained not less than 10 specimens of mussel and 5 specimens of Cystoseira. The bodies of mussels and algae of the same size were dried, preserved in desiccators, and reduced to a constant weight immediately before analysis to determine the concentrations of metals.

Method of Calcining the Biosamples and Analyzing the Heavy Metal Concentration. The samples were prepared for analyses using the method of dry calcination at a maximum temperature of 450°C and subsequently dissolved in hydrochloric acid. The concentration of Cu, Zn, and Pb in the solutions was measured by the atomic absorption method in Perkin-Elmer 403 and AAS-1 (Karl Zeiss, Jena) devices. The preliminary calibration of the devices showed a satisfactory convergence of the data. The concentrations of metals in the biosamples were calculated with respect to the weight of the dry residue.

Concentration Ranges of Cu, Zn, and Pb in the Samples. The data on the concentrations of Cu, Zn, and Pb in the bodies of mussels from the average size group having a shell length of 40-45 mm (mean age of approximately 2 years) are shown in Table 1. Stations 1, 2, and 3 were located on the Crimean coast and Stations 4 and 5 were located on the Caucasian coast. The lowest concentrations for all metals were found in the samples from Station 3, located in the region of the Karadagskaya biostation of the Institute of Southern Seas Biology of the AN USSR. This formed the basis for selecting this station as the "basal" station and conducting the corresponding experiments in the prognostic monitoring program.

TABLE 1. CONCENTRATIONS OF METALS IN BLACK SEA MUSSELS

	Concentrations, µg/g dry weight		
Station	Zn	Cu	Pb
1	250	5.93	2.76
2	179	5.36	2.94
3	150	7.60	0.92
4	166	9.27	3.17
5	231	31.90	7.89

The range of concentrations of Cu in the samples of marine algae collected on the Crimean and Caucasian coasts varied within the limits of 2.0 to 40.0; from 10.0 to 60.0 for Zn, and from 3.0 to 50.0 µg/g dry residue for Pb.

Changes in Metal Concentrations in Samples as a Function of Size (Age). The concentrations of Cu and Zn in M. galloprovincialis living in one colony were not a function of the size of the shells in the broad range of size groups considered (Table 2). The high concentrations of Pb in the first two size groups and Zn in the last two were apparently due to the corresponding changes in the concentrations of these metals in the marine environment.

TABLE 2. CONCENTRATION OF METALS IN MUSSELS AS A FUNCTION OF SHELL LENGTH

Length of shell, mm	Concentration, µg/g dry weight		
	Cu	Zn	Pb
20-26	8.64	269	20.8
30-32	6.62	210	19.4
35-38	6.39	226	3.5
42-46	5.93	250	2.8
50-53	5.87	218	3.9
53-56	6.11	214	4.1
58-60	6.91	256	3.3
67-68	5.01	457	4.3
74-78	6.34	387	4.6

We selected two size groups of mussels with shell lengths of 40-45 and 60-70 mm for subsequent study in the diagnostic monitoring program.

The changes in the concentrations of Cu, Zn, and Pb in the samples of macrophytes as a function of the length of the axial stipes of the algae are shown in Table 3. Algae with axial stipe length of 20-25 cm were selected for the work done in aquarium conditions. The age of the plants was determined according to the method of Sabinin and Schapova (1954). Based on this method, Thallophyta with stipe length of 20-25 cm were approximately one year of age.

TABLE 3. CONCENTRATIONS OF METALS IN SAMPLES OF *Cystoseira* AS A FUNCTION OF LENGTH OF THE AXIAL STIPES

Length of axial stipes, cm	Concentration, µg/g dry weight		
	Cu	Zn	Pb
20	10.3	39.8	8.0
100	5.4	29.4	4.7

Prognostic Monitoring Program

Experimental studies must be conducted under rigorously controlled conditions to determine the limits of accumulation and elimination of Cu, Zn, and Pb by monitoring organisms. The experiments should first concern the following question: With what range of concentrations of metals in the environment can the organisms be reliable monitors? The answer could serve as the basis for developing standard prognostic programs for an organized network of diagnostic monitoring stations.

Mussels and Cystoseira of the same size were selected and acclimatized in flowing marine aquaria for approximately 10 days. The experiments involving fixed additions of solutions of Cu, Zn, and Pb salts were conducted in 30-liter plastic tanks; the water was changed twice a day. A fixed number of specimens of the same size was exposed to different concentrations of the metals; when the water was changed samples were taken from 5 specimens of mussel and 3 Cystoseira plants. Seventy-two hours later, the remaining mussels and plants were placed in special nets, carried out to sea 100 m away from shore and lowered to a depth of 1 meter below the surface. Samples were taken twice a day for the next three days to determine the limits of elimination of the metals. Figure 4 shows a diagram of the changes in the concentration of metals in the samples of mussels and Cystoseira as a function of the concentration in the sea water. The columns in the diagram indicate the maximum values for the concentrations of metals in the mussels and plants. As the diagram shows, mussels and Cystoseira accumulate the metals in direct proportion to their concentration in the water. The data from this brief experiment did not permit establishment of any correlations for this process.

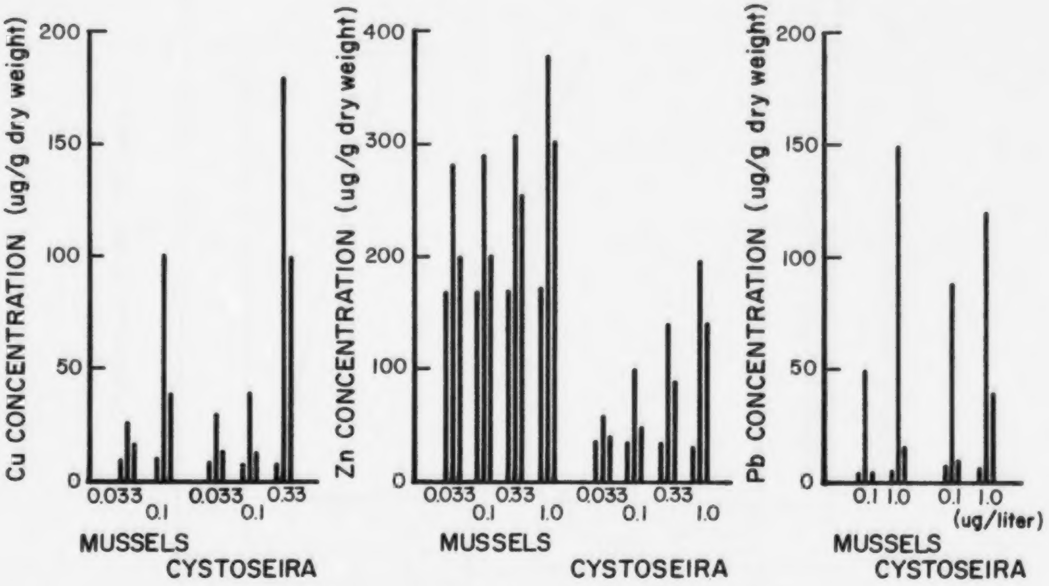


Figure 4. Diagram of changes in the concentrations of copper, zinc, and lead in mussels and Cystoseira as a function of the concentration of metals in the water (first column: controls; second column: 72 hours after accumulation; third column: 72 hours after removal).

The results of these experiments allowed us to establish the range of concentrations within which mussels and Cystoseira could be reliable monitoring organisms for Cu, Zn, and Pb on the coast of the Black Sea. It is evident that the level of accumulation at which the organisms preserve their basic vital functions is the upper limit of this range. In the course of the experiments, the minimum concentrations of copper, zinc, and lead exceeded the mean values for the concentrations of these metals found (based on Patin's data, 1977) in the water of the Black Sea (Cu 4 μg/liter; Zn 30 μg/liter; Pb 3 μg/liter) by no more than 10 times, and the maximum concentrations did not exceed 10 mg/liter. Keeping the mussels and algae in sea water to which a 10 mg/liter concentration of lead had been added for three days caused some inhibition of vital functions, but did not result in death, while a concentration of 1 mg/liter of copper resulted in a 50% death rate in the same period. Keeping mussels and algae in water containing a concentration of 10 mg/liter of zinc for three days did not have any negative effect on the vital activity of the algae, but killed isolated specimens of the mollusks and resulted in general suppression of their vital functions (disorders in the closing function of the adductors and detachment of the byssus to the substrate).

Figure 5 presents the data on the accumulation of copper and the survival rate of the mollusks and algae as a function of the concentration of Cu in the water. We found that the mollusks began to die with a concentration of copper

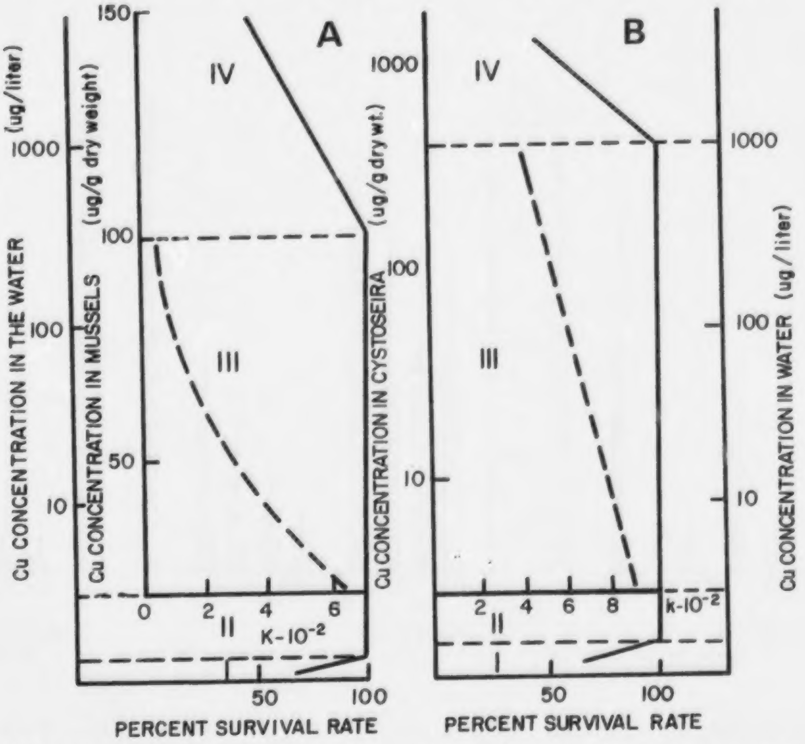


Figure 5. Effect of the concentration of copper in the water and in Mytilus galloprovincialis (A) and the brown alga Cystoseira barbata (B) on their survival and the concentration factor.

of over 100 μg/g (Figure 5A), near the LD_{50} (150 μg/g) for mussels when they were kept in water containing a 1 mg/liter concentration of copper for three days. Keeping Cystoseira in sea water containing copper (1 mg/liter) with a concentration of approximately 300 μg/g in the thallus turned the plant dark, gave it a sharp odor, and killed it.

The data allowed us to determine four basic ranges of concentrations for the metals in water and the corresponding ranges of concentrations in the tissues of the mollusks and algae at which they exhibit certain changes related to their vital activities. The ranges of concentrations, principally characterized by the different mechanism of action of the metals on the organism sampled, are indicated by Roman numerals in Figure 5.

I - The range of a deficit of the metal is characterized by the fact that its concentration in the water is so small that a shortage of the element can result in inhibition of growth and even the death of the animal or plant. This range was hypothetically distinguished, since this situation seldom occurs in a marine environment in natural conditions. The growth curves for unicellular marine algae grown in environments poor in microelements were determined in experimental conditions, but there are no data on conducting analogous experiments on macrophyte and mollusks in the literature.

II - The range of biogeochemical response corresponds to the basal level of the concentration of the metal in the organisms and is exclusively a function of the ecologic conditions. This range is related to fluctuations in the concentrations of metals in the marine environment determined by the hydrochemical and geochemical conditions in the living place. The investigation of the elemental composition of the organisms in this range of concentrations belongs to the field of biogeochemical studies.

III - The monitoring range reflects the response of the organisms to changes in the concentration of metals in the water which are primarily of anthropogenic origin. In this range of concentrations, the monitoring organisms are exposed to an effect responsible for certain biological effects which are a function of the concentration of the metal in the marine environment, the duration of the effect, and the form of its existence. The greatest difficulties are related to establishing the upper threshold of the range of concentrations which is actually the lower threshold of the appearance of a toxic effect. In our work, we conducted brief acute experiments to establish this range. We shall subsequently specify the upper limit of the monitoring range by conducting longer experiments.

IV - The range of the toxic effect corresponding to manifest disorders in the basic physiologic functions of the organisms which lead to their death.

In the proposed classification of concentration ranges in monitoring organisms, the monitoring range for copper in mollusks is from 20 to 100 μg/g of dry residue and is from 3 to 400 μg/g for Cystoseira. The zinc monitoring range for mollusks is within the limits of 150-500 μg/g of dry residue when the concentration of Zn in the water varies from 30 to 3,000 μg/liter. Since almost no lead is detected in the tissues of mollusks with a concentration of

3 μg/liter in the marine environment, we can assume that the lead monitoring range is within the limits of 0-300 μg/g of dry residue when the concentrations in the marine environment varies from 3 to 10,000 μg/liter.

We intend to determine the rate of accumulation and elimination of metals by selected monitoring organisms, to determine the biologic nature of these processes based on elementary and biochemical studies of different organs, and to extend the list of heavy metals which will be investigated in subsequent, more prolonged studies based on the prognostic monitoring program.

SUMMARY

The paper deals with a general scheme for monitoring the environment, biological monitoring, and the monitoring of the pollution of marine biota. The state of coordination of various types of monitoring in the hierarchic structure of monitoring the environment has been shown, the interaction of different research trends and the place of our investigation in this structure which is devoted to developing a program for monitoring the pollution of macrobenthos by heavy metals.

The results of the experiments in this monitoring program are given in the second part of the report. A study has been made of mussels (Mytilus galloprovincialis) and brown algae (Cystoseira barbata) collected in the littoral zone of the Crimea and Caucasus. The samples have been studied morphometrically, weighing the parameters and concentration of Cu, Zn, and Pb by atomic absorption spectrophotometer. At the first stage, reconnaissance observations were conducted within the framework of diagnostic monitoring which made it possible to estimate the change in the concentrations of metals depending on the size (age) of the organism, to determine the ranges of concentrations of Cu, Zn, and Pb in samples collected at different stations, as well as to choose a "basic" station for conducting experiments regarding a program of forecasting. These experiments were made in aquaria of the Institute of Biology of the Southern Seas. Each experiment took six days (three days of monitoring organisms treated in sea water with one of the metals, and after that they were withdrawn and placed in the natural water of a marine bay for three days). These experiments showed the change in the concentration of heavy metals in the tissues of mussels and algae at the stage of accumulation and extermination in relation to the concentration of metals in sea water. The limits of concentrations were determined at which these organisms may be used as reliable monitors of Cu, Zn, and Pb in sea water of the littoral zone of the Black Sea.

REFERENCES

Berner, L., J. H. Martin, J. McGowan, and J. Teal. 1972. Sampling marine organisms. *In* Marine Pollution Monitoring: Strategies for a National Program. Deliberations of a workshop held at Santa Catalina Marine Biology Laboratory of the University of Southern California, Allan Hancock Foundation, October 25-28, 1972.

Gasilina, N. K., and F. Ya. Rovinskiy. 1977. A national system for monitoring the environment in the USSR. *In* Proceedings of the First Soviet-English Symposium. Environmental Monitoring, Gidrometeoizdat Press, Leningrad.

Gerasimov, I. P. 1977. Scientific principles of environmental monitoring. Ibid.

Goldberg, E. D. 1975. The mussel watch--a first step in global marine monitoring. Mar. Pollut. Bull. 6(6).

Izrael', Yu. A. 1977. The concept of monitoring the state of the biosphere. *In* Proceedings of the First Soviet-English Symposium. Environmental Monitoring, Gidrometeoizdat Press, Leningrad.

Martin, J. G. 1979. The effect of metals on the marine environment. *In* Man and the Biosphere, 3rd ed., MGU, Moscow.

Patin, S. A. 1977. Ecologic toxicology and biogeochemistry of pollutants in the world's oceans. Doctoral Dissertation, Moscow.

Sabinin, D. A., and T. F. Schapova. 1954. Growth rate, age, and production of *Cystoseira barbata* in the Black Sea. Tr. In-ta okeanologii, 8.

Simonov, A. I. 1977. Oceanographic aspects of the pollution of seas and oceans. *In* Proceedings of the First Soviet-English Symposium. Environmental Monitoring, Gidrometeoizdat Press, Leningrad.

EFFECTS OF LIQUID INDUSTRIAL WASTES ON ESTUARINE ALGAE, PLANTS, CRUSTACEANS, AND FISHES

by

Gerald E. Walsh and Richard L. Garnas
Environmental Research Laboratory
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

ABSTRACT

A chemical fractionation scheme using ion-exchange resins for separation of organic and inorganic constituents of complex industrial wastes has been developed for use with bioassays in order to identify the toxic components of such wastes.

Grass shrimp (*Palaemonetes pugio*) and sheepshead minnows (*Cyprinodon variegatus*) were not as good indicators of possible effects of complex wastes on estuarine organisms as the diatom, *Skeletonema costatum*, in bioassays performed at our laboratory. The diatom was affected by wastes in either of the following three ways: stimulation, inhibition, or stimulation at low concentrations but inhibition at higher concentrations. Toxicity to algae correlated with toxicity to the mysid, *Mysidopsis bahia*.

Chemical fractionation with appropriate biological testing provides a means of estimating a more complete potential effect of an effluent on receiving waters. Effects of growth stimulators, for example, can be masked by toxicants in whole waste.

We conclude that chemical fractionation is required for comprehensive analysis of possible effects of complex industrial wastes on estuarine organisms, and that fractionation should be coupled to bioassays that use at least an alga and a crustacean as test organisms.

INTRODUCTION

The possible impact of increasing industrialization along coastlines on marine ecosystems is great because industrial plants often emit large volumes of liquid effluents that contain bioactive organic and inorganic substances. Also, because the population of coastal areas is increasing, the volume of sewage and municipal wastes discharged will increase, thus placing a double burden upon marine ecosystems.

Very little is known about effects of complex liquid effluents on aquatic organisms. Data that describe effects are lacking for most substances found in such wastes, and even when effects are known, it is impossible to predict how a bioactive substance will behave in the presence of other substances. In order to estimate the potential effect of a complex waste, chemists and biologists must work together so that bioactive substances can be identified and their effects measured.

The purpose of the work reported here was to evaluate the potential hazard of liquid wastes from industrial plants and one sewage treatment plant to aquatic organisms. The report discusses use of chemical analyses and bioassays to evaluate potential impacts of complex wastes on estuarine algae, seagrass, mysids, shrimp, and fish.

METHODS

Liquid waste samples were collected from the outfalls of industrial plants and a sewage treatment plant located near estuarine areas and shipped by air, either in glass jars under ice in insulated containers or uniced in non-toxic polyethylene containers. Samples were collected and shipped in the morning and received in the afternoon, when tests were begun.

Upon receipt, each waste was examined for color, odor, suspended matter, pH, and salinity. It was then divided between the chemistry and bioassay laboratories for detailed analyses.

Chemical Methods

A fractionation scheme for chemical analysis was devised to be used in conjunction with bioassays (Figure 1). If a complex waste was toxic, it was passed through an XAD resin column that adsorbed dissolved organic matter. Organic compounds were described from the resin with acetone and used in bioassays. If the organic fraction was toxic, it was subfractionated into compounds that were extractable with acetone under acidic, basic, and neutral conditions.

The liquid that passed through the XAD resin column was considered to be the inorganic fraction. If this fraction was toxic, it was subfractionated into the heavy metal portion by use of a Dowex[1] strongly basic anion exchange resin, and into the non-heavy metal portion by use of a Dowex strongly basic cation exchange resin.

Organic and inorganic fractions and subfractions were reconstituted in artificial seawater before use in biological tests.

---

[1] Dow Chemical Company, Midland, Michigan. Mention of trade names does not constitute endorsement by the Environmental Protection Agency.

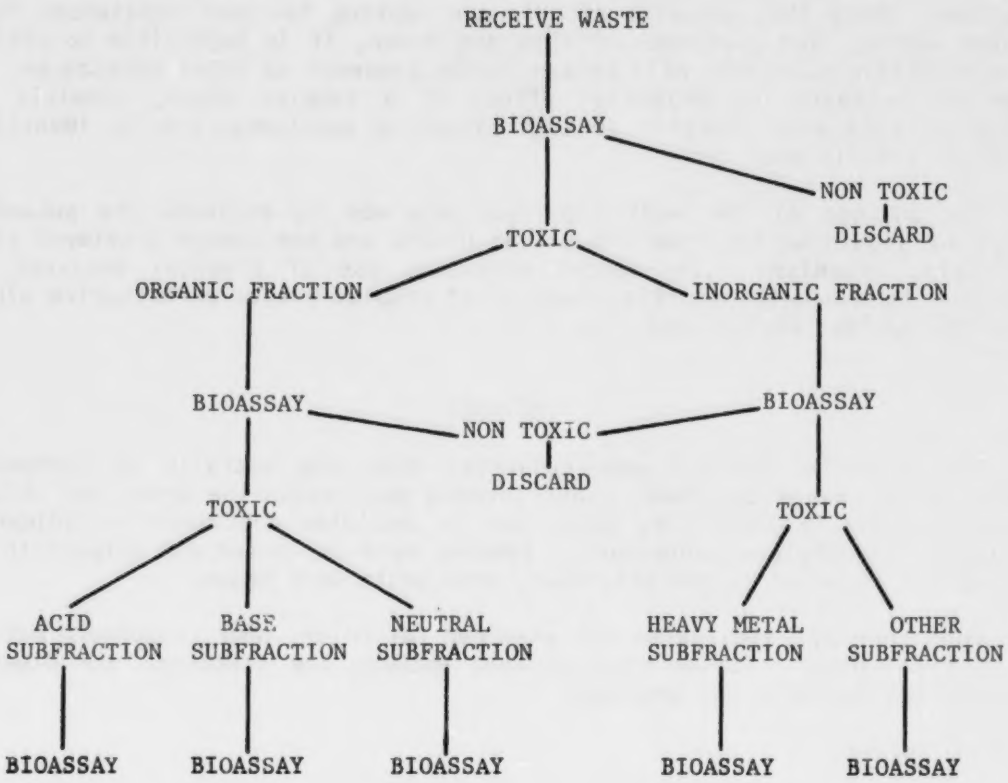

RECEIVE WASTE
BIOASSAY
NON TOXIC
DISCARD
TOXIC
ORGANIC FRACTION
INORGANIC FRACTION
BIOASSAY
NON TOXIC
BIOASSAY
DISCARD
TOXIC
TOXIC
ACID SUBFRACTION
BASE SUBFRACTION
NEUTRAL SUBFRACTION
HEAVY METAL SUBFRACTION
OTHER SUBFRACTION
BIOASSAY
BIOASSAY
BIOASSAY
BIOASSAY
BIOASSAY

Figure 1. Integrated chemical and biological tests for analysis of complex industrial waste.

Biological Methods

Bioassays were used to direct the course of chemical fractionation according to the flow-chart in Figure 1. For S. costatum, P. pugio, and C. variegatus methods for bioassay are given by EPA (1977), and for mysids by the EPA Ocean Disposal Bioassay Working Group (1978). Toxicity to animals is expressed as the LC_{50}, which is the calculated concentration that would be lethal to 50% of the exposed animals.

1. Skeletonema costatum, a chain-forming diatom, is known to respond to toxicants and growth-stimulating substances. Growth was measured as increase in absorbance at 525 nm and by cell counts. In this report, EC_{50} is the calculated concentration that would inhibit growth by 50% as compared to control growth; the SC_{20} is the calculated concentration that would stimulate growth by 20% as compared to control growth.

2. *Mysidopsis bahia*, a mysid, was used to test for survival of animals in some bioassays.

3. *Palaemonetes pugio*, the grass shrimp, was used to test for survival in textile industry waste.

4. *Cyprinodon variegatus*, the sheepshead minnow, was also used in survival tests with textile industry wastes. Only juveniles were used in our tests.

5. *Zostera marina*, a seagrass from Chesapeake Bay, was shipped to the U.S. EPA Environmental Research Laboratory, Gulf Breeze. This species survived in a healthy state for up to three months in our culture room when planted in sand in aquaria.

6. *Thalassia testudinum*, a seagrass that grows in abundance near the Gulf Breeze laboratory, was also used in phytotoxicity tests.

The method of exposure for seagrass used a 4-liter volume reaction kettle fitted with a false bottom. Seagrasses were planted in sand in the false bottom (10 plants per kettle), and the waste was stirred continuously by a magnetic stirrer. Salinity of waste was increased to 30 parts per thousand by adding artificial sea salts (Rila Products, Teaneck, NJ). The waste was diluted with artificial seawater prepared with deionized water and concentrations tested were 25, 50, 75, and 100% water. Controls were prepared from artificial sea salts and deionized water. Plants were considered dead when the leaves turned brown and began to disintegrate.

A new computer method to estimate effects of wastes on algal population growth was used (Walsh *et al*., in press). Response of *S. costatum* was estimated as (1) the calculated concentration that would inhibit growth by 50% (EC_{50}) and (2) the calculated concentration that would stimulate growth by 20% (SC_{20}). Stimulation or inhibition of algal growth was calculated by plotting the non-linear regression of absorbance on waste concentration by the equation

$$Y = \frac{A}{1 + B\uparrow (X - C)} - \frac{A}{1 + D\uparrow (X - E)}$$

where A = mean maximum biomass, B = increasing slope, C = waste concentration (increasing slope) where Y = 0.5 x A, D = decreasing slope, E = waste concentration (decreasing slope) where Y = 0.5 x A, X = range of waste concentrations tested, and Y = estimated population density over range of X. Calculations were made on a Digital Equipment Corporation PDP 11/45 computer. The EC_{50} and SC_{20} were calculated from the regression curve.

On the average, algal growth stimulation of approximately 20% above the control value (SC_{20}) can be considered to be a statistically significant increase in growth in these tests. The mean upper 95% confidence interval for algal growth in control cultures was 17% above the control mean growth value in 20 textile and other industrial effluent assays. Therefore, growth stimulation greater than 17% above control was a significant response. To predict a number that represents a statistically significant increase in growth requires that the standard deviation of the predicted value also be considered. For 20 effluent assay analyses of textile and other wastes, 27% predicted growth stimulation that was significant ($\alpha = 0.05$) as compared to growth. This value supports the concept of SC_{20}, which can be used as an index of significant algal growth stimulation in effluent bioassays (Bahner and Oglesby, in press).

RESULTS AND DISCUSSION

Whole Wastes

Animals and Algae. Shrimp and fish are generally more sensitive to individual toxicants such as chlorinated hydrocarbons than algae or plants. A major finding of our work is that algae are better indicators of complex waste bioactivity in static tests than grass shrimp and sheepshead minnows. In a comparative study using 14 wastes from the textile industry, all 14 wastes affected algal growth, whereas only five were toxic to shrimp and fish (Table 1).

TABLE 1. COMPARISON OF RESPONSE OF *Skeletonema costatum*, *Palaemonetes pugio*, AND *Cyprinodon variegatus* TO TEXTILE WASTES (SC_{20} = percentage waste at which growth was stimulated by 20%; EC_{50} = percentage waste at which growth was inhibited by 50%; LC_{50} = percentage waste lethal to 50% of the animals; NE = no effect.)

Waste	*S. costatum*		*P. pugio*	*C. variegatus*
Code	SC_{20}	EC_{50}	LC_{50}	LC_{50}
C	1.50	76	12.8	69.5
N	NE	2.0	26.3	47.5
T	2.00	66	34.5	68.0
W	1.50	50	19.6	37.5
A	--	--	21.2	62.0
B	0.50	NE	NE	NE
F	NE	84	NE	NE
G	2.75	59	2/	3/
K	1.00	79	NE	NE
L	NE	1.5	NE	4/
S	2.25	NE	2/	2/
U	1.50	NE	NE	NE
V	21.75	93	NE	NE
X	0.50	NE	NE	NE
E	--	--	3/	1/
P	NE	9.0	--	--

1/ 10% mortality in 100% waste; 2/ 20% mortality in 100% waste;
3/ 40% mortality in 100% waste; 4/ 50% mortality in 100% waste.

Bioactivity toward algae occurred in three patterns: growth stimulation, growth inhibition, and growth stimulation at low concentrations and growth inhibition at higher concentrations (Figure 2). Results of all three types are common and indicate that industrial effluents are sources not only of toxic materials but also of growth stimulants that may accelerate the eutrophication process.

Data generated from bioassays of whole waste can be used to estimate potential impact relative to other wastes. We compared our data for 14 textile plant effluents to that reported for the same plants with freshwater organisms (Selenastrum capricornutum, Daphnia pulex, Pimephales promelas) (Walsh et al., in press) and found that S. costatum was the most sensitive species in 12 cases. The 14 samples were then ranked according to their potential impact by relating the volume of discharge to response concentration according to the expression:

$$\mathrm{MEU/d} = \left(\frac{100}{\mathrm{EC}_{50} \text{ or } \mathrm{SC}_{20}}\right) \text{(discharge rate)}$$

where MEU/d = million effective units per day. Sample X, which had a low SC_{20} and a high discharge rate, is judged to have the greatest potential for adverse effect upon its receiving water. Waste W, with a low SC_{20} and a low discharge rate, was judged as having a relatively low potential for adverse effect (Table 2).

TABLE 2. RELATIVE POTENTIAL IMPACT, in MEU/d, OF 14 SECONDARY TEXTILE MILL WASTES TESTED WITH FRESHWATER AND ESTUARINE ALGAE, CRUSTACEANS, AND FISHES

Waste	Discharge $m^3/d \times 10^3$	Most sensitive organism	Response	Percentage waste Lowest Value	MEU/d $\times 10^{-3}$
B	4.5	S. costatum	SC_{20}	0.5	900
C	3.8	S. costatum	SC_{20}	1.5	253
F	7.6	D. pulex	EC_{50}	81.7	9
G	7.6	S. costatum	SC_{20}	2.8	271
K	9.5	S. costatum	SC_{20}	1.0	950
L	2.8	S. costatum	EC_{50}	1.5	187
N	3.8	S. costatum	EC_{50}	2.0	190
P	4.5	S. costatum	EC_{50}	9.0	50
S	4.7	S. costatum	SC_{20}	2.3	204
T	2.3	S. costatum	SC_{20}	2.0	115
U	1.1	S. costatum	SC_{20}	1.5	73
V	3.0	D. pulex	EC_{50}	9.4	32
W	1.0	S. costatum	SC_{20}	1.5	67
X	9.8	S. costatum	SC_{20}	0.5	1,960

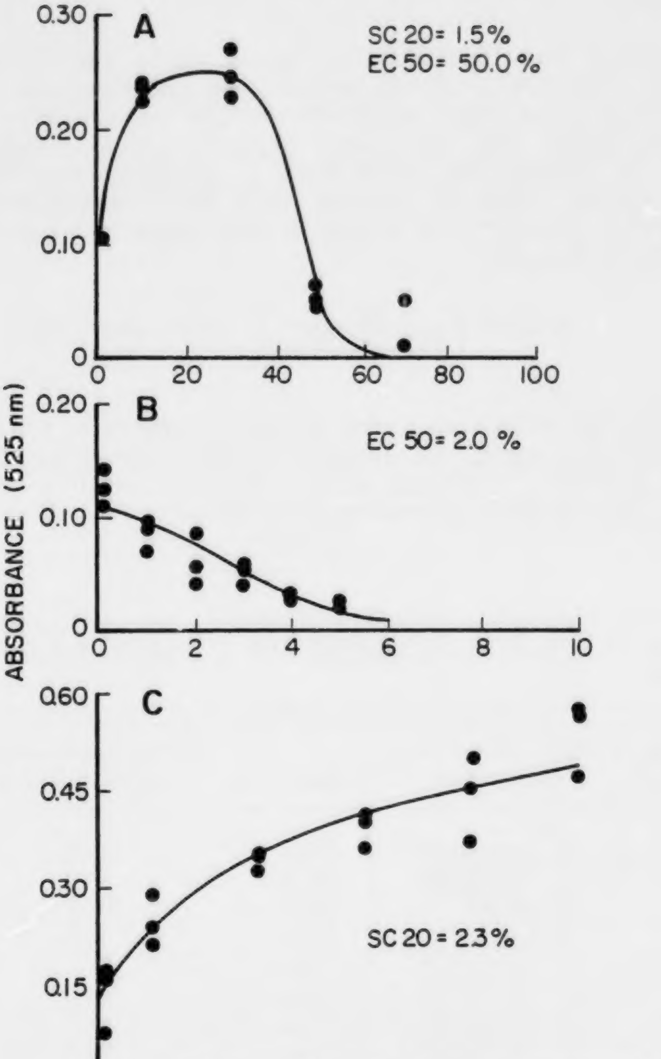


Figure 2. Effects of three textile mill wastes on growth of _Skeletonema costatum_. EC_{50} = calculated concentration that would inhibit growth by 50%; SC_{20} = calculated concentration that would stimulate growth by 20%.

Algal data are not widely used for setting water quality standards because algae usually are not as sensitive to individual organic pollutants as invertebrates or fishes. Our data show that S. costatum was the most sensitive organism to most of the complex wastes tested. It was especially valuable because such wastes often contain nutrients and toxicants that do not affect the test animals. We suggest that algal tests should be an integral part of testing programs for complex wastes.

Seagrass. Seagrasses are important components of estuarine ecosystems, and we have just begun a long-term study of effects of pollutants on them. In preliminary experiments, seagrasses were exposed to six waste effluent samples, and three of the samples were toxic to them (Table 3). Seagrasses are declining in many parts of the world, mainly in industrialized countries, but this is the only study that we are aware of that demonstrates direct toxicity of industrial wastes to them.

TABLE 3. EFFECTS OF INDUSTRIAL WASTE ON SEAGRASS AFTER THREE WEEKS OF EXPOSURES

Industry	Species	Effect
Creosoting	T. testudinum	Not toxic
Chemical	T. testudinum	All plants dead in all concentrations
Steel	T. testudinum	(1) Two plants dead in 50% waste (2) Three plants dead in 75% waste (3) Four plants dead in 100% waste (4) All surviving plants in 50, 75, and 100% waste were chlorotic
Steel	T. testudinum	Not toxic
Chemical	T. testudinum	Not toxic
Sewage Plant	Z. marina	(1) All plants exposed to 25 and 50% waste were degraded (2) All plants exposed to 75 and 100% waste appeared dead

Field Studies. We received 11 waste samples that were tested in flow-through bioassays in the field. Field bioassays done in a mobile laboratory demonstrated that four were toxic and seven were non-toxic to shrimp and fish. In laboratory bioassays, seven were toxic and four were non-toxic to algae, shrimp, and fish. Algae responded to all, either by growth stimulation or growth inhibition. We recommend, therefore, that expensive and time-consuming on-site bioassays need not always be done. Laboratory tests identify bioactive wastes and can be used effectively for screening waste outfalls.

Fractionated Wastes

When toxic whole wastes were fractionated according to the scheme given in Figure 1, toxicity was found to be in the organic or inorganic fractions, seldom in both. Table 4 gives results from a few industries whose wastes were tested with *S. costatum* and *M. bahia*. Alga and mysid were generally useful in identifying toxic fractions and non-toxic wastes.

TABLE 4. RESPONSES OF MYSIDS (*M. bahia*) AND ALGAE (*S. costatum*) TO FRACTIONS OF INDUSTRIAL WASTES (+ = toxic effect; - = no toxic effect)

Industry	Mysid	Algae	Toxic Fraction
Gunpowder	-	-	-
Titanium oxide	+	+	Heavy metals
Aliphatic amines	-	-	-
Oil refinery	+	+	Neutral organic
Tall oil products	+	+	Neutral organic
Phosphoric products	+	-	Heavy metals
Nylon	-	-	-
Carpeting	+	+	Heavy metals
Citric acid	-	-	-
Synthetics	-	-	-
Carpeting	-	-	-
Amine products	+	+	Heavy metals
Textiles	+	+	Heavy metals
Paper products	+	+	Neutral and acid organics
Unbleached paper	-	-	-
Wire and Rod	-	-	-
Municipal STP	+	+	Heavy metals

Fractionation followed by bioassay simplifies identification of toxic components in complex wastes because only the toxic fraction needs to be analyzed chemically. For example, waste from a titanium oxide plant was found to be highly toxic, and all toxicity was present in the heavy metal subfraction. No other subfraction was toxic. Heavy metals analysis of the heavy metal subfraction revealed very high concentrations of some metals (Table 5) and it was recommended that the industrial plant apply control technology to the metals portion of its effluent. In that way, only a single fraction of the effluent needed to be treated and highly expensive treatment of whole waste was avoided.

It is important to test fractions of an effluent because effects of bioactive substances may not be detected in whole waste. Effects of three wastes and their fraction on *S. costatum* are given in Table 6. Whole waste was toxic in each case, and none stimulated growth. The organic fractions had no effect on algal growth. Toxicity of each waste was in cationic subfraction of the inorganic fraction.

TABLE 5. HEAVY METALS ANALYSIS OF A TITANIUM OXIDE PLANT WHOSE EFFLUENT TOXICITY WAS IN THE HEAVY METALS SUBFRACTION (Analysis by inductively coupled argon plasma method)

Element	Concentration, µg/L
Ti	23,000
V	3,800
Cd	40
Cr	1,800
Cu	70
Zn	625
Zr	200
Fe	260,000
Al	18,000

TABLE 6. EFFECTS OF INDUSTRIAL WASTES ON GROWTH OF Skeletonema costatum (EC_{50} = calculated percentage that would inhibit growth by 50%; SC_{20} = calculated percentage that would stimulate growth by 20%. STP sewage treatment plant)

	Whole Waste		Inorganic Fraction		Cations		Anions	
Category	EC_{50}	SC_{20}	EC_{50}	SC_{20}	EC_{50}	SC_{20}	EC_0	SC_{20}
Chemicals	8.2	none	9.6	none	16.0	none	none	1.0
Fibers	15.4	none	22.1	0.4	16.5	none	none	0.9
STP	14.0	none	30.9	none	20.0	none	none	5.6

For the fiber plant, the inorganic fraction was highly stimulatory (SC_{20} = 0.4%) when separated from the organic fraction. The anionic subfraction of each waste was highly stimulatory to algal growth when separated from the heavy metals. These observations show that effects of growth stimulants may not be detected when toxicants or other substances are present. In receiving waters, where toxicants and stimulants may be separated spatially and transformed biologically or chemically, algal growth promotion, resulting from eutrophic conditions, could be the most significant effect of a waste stream.

We suggest that chemical fractionation and algal and other bioassays should be done in order to assess potential effects of a liquid waste stream on receiving waters.

SUMMARY

Industrial effluents are major sources of pollution in aquatic ecosystems. Chemical analyses alone cannot predict effects of such complex wastes (Walsh *et al.*, in press), and it is necessary that bioassays be done on algae, plants, and animals if the potential impact of a waste is to be estimated. This is especially true when both algal growth stimulators and toxicants are present in a single waste.

Bioassays of whole complex-waste effluent is useful for regulation by law enforcement agencies. However, effects of stimulants or toxicants may be reduced by the presence of other substances. Since components of bioactive complex wastes may become separated in receiving water, their organic and inorganic components should be fractionated in the laboratory and bioassays on fractions should be performed to estimate the total potential bioactivity and to identify the bioactive substances. After these substances are identified, technology may be applied to the industrial process for their removal.

REFERENCES

Bahner, L. H., and J. L. Oglesby. Models for predicting kepone accumulation and toxicity in laboratory exposures and natural ecosystems. *In* Environment Risk Analysis for Chemicals, R. A. Conway (ed.) Van Notrand Co., New York. In press.

U.S. Environmental Protection Agency. 1977. IERL-RTP procedures manual: level 1. Environmental assessment biological tests for pilot studies. EPA-600/7-77-043. Industrial Environmental Research Laboratory, Research Triangle Park, North Carolina.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the ocean disposal permit program. EPA-600/9-78-010. Ocean Disposal Bioassay Working Group, Cincinnati, Ohio.

Walsh, G. E., W. B. Horning, and L. H. Bahner. Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans, and fishes. Environ. Pollut. In press.

ECOLOGIC ASPECTS OF USING CHEMICAL AGENTS FOR ELIMINATING THE RESULTS OF OIL SPILLS IN THE OCEAN

M. P. Nesterova
P.P. Shershov Oceanology Institute
USSR Academy of Sciences

Eliminating oil pollution in the ocean is one of the major ecologic problems in environmental protection.

Petroleum and petroleum products are the most common pollutants of the world's oceans. It is sufficient to say that emulsified and dissolved petroleum products have been found in half of the 28,000 samples taken in different areas of the Pacific and Indian Oceans by ships from the AN SSSR Institute of Oceanology. During an international MOK and VMO experimental project to detect oil films on the surface of the seas and oceans.

Of the 3500 miles covered in the northwest Pacific, 315 contained an oil film, as determined by a remote procedure conducted from the side of a ship. Studies (Simonov et al. 1974) have shown there is significant pollution from petroleum products in the northern part of the Atlantic Ocean. This has been confirmed by the studies of the AKADEMIK KURCHATOV research ship (January-June 1978) in the Sargasso Sea and in the region of the Gulf Stream, where 83 trawl nets took samples for determining the concentration of tar lumps; only six of the 75 trawl nets contained no tar lumps in the Sargasso Sea. Eight trawl nets were used for cutting through the Gulf Stream, and only two contained tar lumps.

The external appearance of the lumps differed strongly; some were sticky, fairly fresh petroleum products, but most were dense, structurized coagulates; there were brown films in some places. Young goose barnacles, Bryozoa, algae and other organisms were often found inside the lumps

The presence of different types of oil and petroleum products in the waters of the world's oceans indicates the predominance of accumulation over biochemical degradation.

Petroleum pollution must have an effect on the physical, chemical and biologic processes of the seas and oceans. The destructive effect of oil on hydrobionts is well known. Reports of the effect of oil on the inhabitants of the ocean began to appear in the literature in the 19th century. Many investigators probed this question at the beginning of the 20th century, and extensive literature appeared. At the beginning of the '70s, Nelson-Smith reported more than 800 studies on the different aspects of the biologic effect of oil (Nelson-Smith 1970, 1972). The oil film on the surface of the ocean also disturbs exchange of energy, heat, moisture and gases between the ocean and the atmosphere. We found experimentally that evaporation had decreased to 45 t after one hour in one square mile of ocean surface, i.e., by almost two times. The presence of an oil film over a large surface can apparently also affect weather conditions (Buynitskiy 1976).

The marine fleet is the basic source of petroleum pollution of the world's oceans (approximately 33%), and a particularly important fact is that this pollution occurs in normal operating conditions. Eliminating this source of pollution would only be possible by improving technological processes and control methods. However, even the introduction of new technology and improved operating conditions cannot totally prevent accidents and catastrophes similar to the AMICO-CADIZ and hundreds of others. Oil spills, sometimes very large ones, can naturally also occur in underwater fields. Accidents in offshore drilling and tankers are the most dangerous and seriously damage the ecological systems in individual regions of the world's oceans. It has been found that 20-30% of the total pollution of the marine environment is caused by oil spills.

The oil which enters the sea as a result of spontaneous spillage, and the effect of waves and wind result in the formation of films of different thicknesses on the water surface. This is a primary, unstable form of oil in sea water, since it is constantly changing under the effect of the surrounding environment. First, the volatile components of petroleum and petroleum products evaporate very intensively under the effect of strong winds, swells and temperature, and during evaporation crude oil can lose up to 50% of its components, diesel fuel up to 75% and mazut from 5-9%. Evaporation begins when the oil first enters the ocean, proceeds at a decreasing rate, and then becomes insignificant and is almost completely over in a few days. However, it is not true that evaporation stops the toxic effect of the oil on ecologic associations. Conversion of the components of the spilled oil to the gas phase results in pollution of the atmosphere, primarily the layer adjacent to the water, and does not prevent the components from re-entering the water. Very little spilled oil is dissolved in sea water (up to 5%); the largest amount undergoes emulsification and dispersion. Depending on hydrodynamic processes and meteorologic conditions, physicochemical dispersion can rapidly (in a few hours) eliminate 15% of the spilled oil. Although dispersion accelerates biochemical oxidation, the capacity of a body of water for self-cleaning is not infinite. And, as the emulsified and dispersed oil decomposes, compounds which are more toxic to the inhabitants of the ocean than the oil itself can be formed. In the absence of turbulence, dispersed oil can again form oil films on the ocean surface. Dispersed oil can be absorbed on mineral suspensions and plankton and enter the deeper layers of the ocean with these carriers.

Dispersed oil is particularly rich in surface-active substances and forms "oil in water" emulsions; however, in many cases where oil accumulates in large quantities on a limited surface, it forms an emulsion in mixing with the water, "water in oil," since the high-molecular compounds it contains (tars, asphalts, etc.) stabilize emulsions of this type. Such highly viscous cross-linked formations ("chocolate mousse," tar lumps) can remain on the water surface for a long time; when the mineral suspension condenses and is absorbed, they can sink to the bottom or be tossed on shore, causing considerable damage both to benthic organisms and coastal fauna and flora. Some of the methods proposed for eliminating oil spills are based on this principle, i.e., sinking the oil with specially introduced mineral hydrophobic additives (hydrophobic sand, carbonaceous materials, etc.). Naturally, this method is not suitable.

The quantitative ratio of migratory forms of petroleum hydrocarbons in the different regions of the seas and oceans also varies within very broad limits, but can be used as some indication of the processes which result from an oil spill. For example, the 26th passage of the research vessel AKADEMIK KURCHATOV in the central part of the Baltic Sea in 1978 revealed that 3.4% of petroleum hydrocarbons was found in the film, 15.6% in residue and 81% in the water; 12% was dissolved, 10% was in a colloidal-disperse state, and 78% was emulsified. This indicates redistribution of the petroleum hydrocarbons under the effect of the environment (a significant effect) even when there were no changes in the chemical composition of the oil.

However, conversion of oil from one form to another cannot be considered utilization or elimination of the consequences of oil spills in the sea. Each migration form of petroleum hydrocarbons will have a negative effect on the ecology of the region. For this reason, methods and agents to achieve maximum removal of the oil pollution resulting from spills in the marine environment are necessary. The AN SSSR P.O. Shershov Institute of Oceanology adhered to these concepts when developing methods and agents for eliminating the consequences of oil spills.

Chemical agents are being developed to enclose an oil spill to prevent it from spreading, and methods and agents for removing the oil and increasing the natural biochemical decomposition are being devised. An agent has been created that involves forming a dense barrier of the foam-plastic type which prevents a spill from spreading and simultaneously keeps the oil from the surface of the water. After mechanical removal from the surface of the water, the foam-plastic could be squeezed out and reused as a sorbent.

In cooperation with the L'vov Polytechnic Institute, oil sorbents based on plant, mineral and synthetic substances have been developed and investigated. One of the basic requirements for such substances is unsinkability and the potential for recovering the oil. For example, some plastic foams based on complex polyesters which absorbed an amount of oil 18-20 times greater than their weight in 5 minutes have been tested in the laboratory; oil occupied 90% of their volume, and they could be used many times (Anufriyeva and Nesterova 1976). A sorbent was prepared on a mineral base from distended hydrophobic volcanic rock-perlite, which can be used for production of bitumen and other construction materials after the petroleum is absorbed from the surface of the water.

The tests on hydrophobic perlite and thermally treated peat showed that they could clean an area of water in a port of 98-99% artificially produced oil pollution. After most of the oil has been collected mechanically (if a large quantity is involved), the relatively thin residual film is treated with substances which can disperse and convert it to a thin dispersion (emulsion of the "oil in water" type). By eliminating the previously dense oil film, oxygen exchange between the water and the atmosphere is restored and gradual, natural biochemical oxidation of the highly dispersed, isolated droplets of oil suspended in the water layer begins. If the dispersing agent has a high enough stabilizing capacity with respect to these drops and persists even when the initial dispersion is greatly diluted by the water, and if it is economically practical and--more important--not toxic to marine organisms, then this method of eliminating oil pollution should be a very promising and effective method for eliminating the consequences of spills. We used surface active substances as spontaneously active dispersing agents which could destroy a dense oil film and convert it into a stable emulsion when aqueous solutions were introduced in the film.

This condition of spontaneous emulsification is necessary, since the usual methods for forming emulsions, especially highly dispersed emulsions by mechanical fractionation into emulsified drops, are not suitable in this case. For this reason, we used one of the properties of surfactants from the group of nonionic compounds related to their solubility in aqueous and oil (hydrocarbon) phases of emulsions. In contrast with most ionic surfactants whose solubility in any phase is a function of their composition, nonionic surfactants are usually readily soluble in both phases; thus, when they contact an emulsion system, redistribution of the surfactant takes place by diffusion through the interface. This process, as Taubman previously demonstrated (1969), causes strong local decreases in the interphase tension and consequently very pronounced disturbances in the hydrodynamic stability of the easily moved interface. The intensive turbulence in the boundary layers results in the appearance of highly dispersed emulsions of both types; however, only the "oil in water" emulsion survives (the hydrophilic emulsifier only stabilizes emulsions of this type) and a highly dispersed emulsion appears--a microemulsion.

The microemulsion is stabilized by the adsorption layer of the surfactant and is a surface structure which in turn stabilizes the "karli" in the basic microemulsion; as a consequence, the entire system is highly resistant to coalescence.

If we consider the oil film on the surface of the ocean as a separate phase of relatively very low volume, then it follows from the above that introducing an aqueous solution of the corresponding nonionic surfactant with a jet apparently produces the following effect--decomposition of the film into drops which are stabilized to such a degree that the microemulsion formed will retain sufficient stability even with "infinite" dilution with water and the effect of swells and winds.

In basing the emulsion stabilization mechanism on the above, it was necessary to obtain evidence of the possibility of obtaining this effect in actual natural conditions with ordinary dispersing agents with respect to the features of methods of mixing solutions of the agents with the oil film on the surface of the water, the presence of their electrolytes in the water, the effect of strong dilution of the solutions, and several other factors.

The results of laboratory studies and field trials confirmed this possibility.

Dispersing agents have been developed in our laboratory--DN-75 oil disperser and EPN-5 oil-film emulsifier (together with the Ufimskiy Institute of Petroleum Transport and Storage) (Taubman and Nesterova 1977).

The laboratory studies and field trials on these products and on Correxite-7664 (U.S.) and Berol-198 (Sweden) demonstrated the high efficiency of the dispersing agents. In trials in the Baltic Sea, Berol-198 decreased it to 0.06 mg/liter, Correxite-7664 to 1.22 mg/liter and DN-75 to 0.23 mg/liter.

An artificially created petroleum spill (50,000 m^3) in the port of Vladivostok was destroyed by DN-75 in 15 minutes (Nesterova et al. 1977).

Using dispersing agents in an aqueous environment naturally has some effect on the hydrobionts; for this reason, in developing dispersing agents, the Analytic Laboratory of the AN SSSR Institute of Oceanology first concentrated on the toxicologic studies of the products and their constituent parts. The effect of EPN-5 and DN-75 dispersing agents was studied on hydrobionts of different trophic levels both in the Institute of Oceanology and in a number of scientific research institutes in our country: In the Laboratory for Research on Water Pollution of the Saratov Branch of the State NIORKh, the Department of Hydrobiology of the biological faculty at MSU, the Laboratory of Radiation and Chemical Ecology of the VNIRO, the Georgian Branch of the VNIRO and the DVNTs Institute of Marine Biology.

We shall briefly summarize the results of the studies.

The effect of the dispersing agents on aquatic microflora was traced based on changes in the number of saprophytic bacteria in the water containing different concentrations of EPN-5. It was found that EPN-5 undergoes strong bacterial oxidation in the water which facilitates the vigorous development of saprophytic bacteria. The presence of EPN-5 in the water, up to a concentration of 10 g/liter, had no inhibiting effect on multiplication of the bacteria and the number of saprophytic bacteria in the water containing EPN-5 was many times higher than the control. These studies showed that EPN-5 in concentrations over 0.1 mg/liter promotes intensive development of saprophytic microflora.

The effect of the dispersing agents on zooplankton was studied using the water flea. We examined the condition of the water fleas exposed to different concentrations of EPN-5. The results of the experiments showed that 2 mg/liter is the inactive concentration of EPN-5, i.e., EPN-5 is moderately toxic to water fleas.

The effect of solutions of the individual surfactants was investigated--oxyphos and diproxamine 157 in concentrations of 0.1-10 mg/liter--on the survival and growth of the young of a Black Sea isopod. All concentrations of diproxamine 157 tested had no effect on the survival of the animals. The data confirmed the low toxicity of diproxamine 157; the previously established maximum admissible concentration (MAC) for fish breeding waters was 3.2 mg/liter. A 10 mg/liter concentration of oxyphos increased the survival of idothea by 1.3 times and had a positive effect on the growth of the young animals.

The effect of the dispersing agents EPN-5, DN-75, Correxite-7664 and Berol 198, produced by the MoDoKemi AB Co., Sweden, and the surfactant diproxamine 157 and oxyphos on blue-green algae included the following: Inhibition of growth of the algae was observed with a concentration of 100 mg/liter of EPN-5, the nontoxic concentration was 10 mg/liter; for DN-75, these concentrations were one order-of-magnitude higher, respectively. Of the two foreign samples investigated, Correxite-7664 was less toxic than Berol 198, whose toxicity to blue-green algae was the same as the domestic dispersing agent DN-75.

The toxicity of the other surfactant, oxyphos, was the same as that of EPN-5, i.e., a 100 mg/liter concentration caused inhibition of growth in the algae, and a 10 mg/liter concentration was nontoxic. In the range of concentrations investigated, diproxamine 157 had no toxic effect up to a concentration of 10 g/liter.

The studies conducted by the Laboratory of Radiation and Chemical Ecology of the VNIRO showed that unicellular algae were the most sensitive hydrobionts to low concentrations of toxic substances. Their photosynthesis was inhibited by minute quantities of toxic substances which caused no pronounced physiologic anomalies in other organisms. The experiments conducted on Black Sea and Caspian species of unicellular algae showed that a concentration of 100 mg/ liter of EPN-5 caused coagulation of the protoplasm in diatomaceous algae, i.e., actually the death of the cells. The Caspian Ankistrodismus convolutus and the Black Sea Coscinodiscus granii were most resistant to EPN-5; 10 mg/ liter concentrations did not harm these algae. Peridium, green and one of the diatomaceous algae were most sensitive to EPN-5. Only the 0.5 mg/liter concentration of EPN-5 produced optimal results for these three species of algae. Similar studies with the same species of algae were conducted with solutions of Berol 198. The harmless concentrations of this preparation were an order-of-magnitude higher than for EPN-5.

Acute, subacute and chronic experiments were conducted on Chironomid larvae at the Saratov branch of the Gos NIORKh to determine the maximum admissible concentration of EPN-5. The experimental studies showed that the 2.5 mg/liter concentration had no negative effect on metamorphosis of the larvae.

The effect of EPN-5 on fish eggs and larvae was investigated at the same institute. Eggs and larvae of one species of Neopterygii--pike--and one species of cartilaginous ganoids--Russian sturgeon--were used as the test objects in investigating the effect of EPN-5 on fish in the embryonic and postembryonic period of development. The studies showed that the maximum admissible concentration of EPN-5 had no negative effect on the physiologic condition or growth rate of pike larvae and could be considered inactive for pike in the early postembryonic stage of development.

The results of the observations of Russian sturgeon eggs and larvae showed that only a concentration of 0.09 mg/liter had a negative effect on the physiological condition and growth rate of sturgeon and can be considered inactive for sturgeon in the early postembryonic stage of development.

The effect of EPN-5 on adult fish was determined in this year's brood of rainbow trout. The following liminal concentrations which were harmless to the fish based on the following indices were determined: survival 5.6 mg/liter; clinical and pathologic condition 2.8 mg/liter; growth rate 1.4 mg/liter; blood 0.8 mg/liter. Thus, the inactive concentration of EPN-5 for trout is 0.8 mg/liter.

The toxicologic effect of EPN-5 and the dispersing agent DN-75 was investigated at the Georgian branch of the VNIRO on another species of fish--horse mackerel. It was found that DN-75 was less toxic than EPN-5. The MAC for DN-75 was 0.005 mg/liter.

An analysis of the data obtained in studying the effect of EPN-5 on the physiocochemical properties of water, the toxicity for feeding organisms, eggs, larvae and fish showed that fish larvae are the most sensitive component. A maximum admissible concentration of 0.09 mg/liter, the boundary toxicological index, was established for EPN-5 in fish breeding waters.

Studies on the hygienic basis of the maximum admissible concentrations of dispersing agents in bodies of water were conducted at the same time as the studies of the effect of the dispersing agents on hydrobionts. The effect of dispersing agents on the organoleptic characteristics of water and the overall sanitary regime of the reservoirs was investigated.

In generalizing all of the available data on the effect of dispersing agents on hydrobionts, we can conclude that the effect of dispersing agents is both a function of the chemical composition of the substance and the species of biologic object exposed to the dispersing agent. For this reason, only comprehensive studies of the toxicological effect on different levels of the trophic chain will make it possible to recommend chemical agents, particularly dispersing agents, for use on a scientific basis. The scientific and technical studies should be extended with respect to creating agents and methods for eliminating the consequences of oil spills. Correct recommendations and measures for eliminating the consequences of oil spills can only be developed on the basis of fundamental, complex studies of the processes which take place in the sea and on a precise and continuous evaluation of the state of pollution of marine waters.

REFERENCES

Anufriyeva, N. M., and M. P. Nesterova. 1976. Study of the plastic foam polyurethane as an agent for removing oil from the surface of water. Vodnyye resursy 4:149.

Buynitskiy, V. Kh. 1976. Some socioeconomic problems in environmental pollution related to scientific-technologic progress. Vestnik LGU 12:7.

Nesterova, M. P., O. S. Mochalova, and N. M. Antonova. 1977. Chemical agents for eliminating oil pollution of the world's oceans. In Proc. of the 1st Conference of Soviet Oceanologists, Moscow.

Nelson-Smith. 1973. Petroleum pollution of the sea. Gidrometeoizdat, Leningrad.

Simonov, A. I., S. G. Oradovskiy, and A. A. Yushchak. 1974. The current state of chemical pollution of Northern Atlantic waters. Meteorologiya i gidrologiya 3:6-69.

Taubman, A. B., S. A. Nikitina, and V. I. Prigorodov. 1969. The role of quasispontaneous emulsification in stabilizing emulsions. Kol. zhur. (27)2:291-292.

Taubman, A. B., and M. P. Nesterova. 1977. Physicochemical properties of the emulsifying effect of surfactants applied to problems of marine ecology. In Physicochemical principles of using surfactants, Tashkent.

CHEMICAL POLLUTION OF THE FILM LAYER OF THE PACIFIC OCEAN

A. I. Simonov and V. I. Mikhaylov
State Oceanographic Institute
Moscow

The first survey of studies on the chemical pollution of Pacific Ocean waters was completed in 1975. As a result of this effort, Soviet oceanologists were able to draw a number of general conclusions concerning the nature of marine pollution (Oradovskiy _et al_. 1975; Simonov _et al_. 1974). These conclusions have now been confirmed by many studies conducted in different countries which have greatly facilitated determination of strategies for monitoring pollution on national and international levels. We shall mention three basic conclusions. First, it was found that the pollution of the Pacific Ocean, primarily pollution from petroleum hydrocarbons (PH), i.e., petroleum, petroleum products and the products of their decomposition in sea water, unsaturated hydrocarbons, is planetary in nature. Second, the vast role of oceanic circulation in transferring and distributing PH, including in relatively clean areas of the Pacific, for example, in the northern Arctic Ocean, was demonstrated. Third, it was shown that chemical pollution significantly affects primary production, and accelerates decomposition of living organic substances.

However, there were gaps in the survey stage of the studies, basically caused by the lack of methods and technical means for observations and determinations. One of these lacunae consisted of the fact that the role of boundary surfaces (the surface of the ocean, the bottom, the layer of density discontinuity) in the accumulation and evolution of pollutants was not investigated for these reasons.

The results of studies of pollution in the surface layer of the Pacific Ocean by petroleum hydrocarbons and other pollutants are communicated in the present article. The studies were conducted in the Northern Atlantic on scientific-research weather ships from the State Oceanographic Institute in 1976-1977. These studies were important both with respect to the prediction of the level of pollution in ocean waters and with respect to evaluating its effect on the basic physical, chemical and biological processes and energy-heat-moisture and gas exchange between the ocean and the atmosphere.

The observations of pollution in the surface microlayer (abbreviated SML) of the water were conducted in broad areas of the North Atlantic, including the Canary and Northern Tradewind currents, the Gulfstream, the Sargasso Sea and the Northern Atlantic current system. The SML consists of the thin surface layer 100 micrometers thick. Samples were taken in this microlayer with a grid sampler constructed according to the principle proposed by Garret (1972). We note that the samples of water from the SML were taken only in those cases where signs of petroleum hydrocarbons in the visibile frequency range were not observed in the surface.

The grid sampler is a capron sieve with capron thread thickness of approximately 0.2 mm and cell area equal to 1 mm^2. The sieve is mounted on a frame which is not subject to corrosion. Grid samples with a total area of 0.35-1.20 m^2 were used in the study.

In taking samples of water from the SML, special precautions were taken to prevent the ship from becoming a source of pollution. Thus, samples of sea water were taken immediately after the ship stopped and discharge of all waste and ballast water was terminated 15 minutes before sampling began and was not resumed until sampling ended; washing using all types of discharge devices was prohibited.

The water taken from the SML was analyzed for PH, synthetic surfactants (SS) and organochlorine pesticides (OCP) in ship and shore laboratories using the procedures described in the "Handbook of Methods ..." (1972).

Samples of air were simultaneously taken at the individual stations for determination of PH using devices which allow both taking air samples from the atmosphere and preparing an extract in which the concentration of PH is subsequently determined from the side of the drifting ship and during movement. The apparatus consists of the following: a pump device--a VK-I microcompressor with output of 2 liters/min; equipment for removing water vapor from the air samples and eliminating different types of impurities--U-shaped calcium chloride tubes with different filters and extractor vessels. Drexel flasks with atomizers were used for extraction. The microcompressor was installed on the windward side of the ship to exclude the possibility of petroleum hydrocarbon products from the ship itself. The air was pumped by the microcompressor for 0.5-1 hour to concentrate the amounts of PH in the extract; this could be determined by the currently used methods of IR spectrometry and gas chromatography. The air pumped by the microcompressor first passed through a desiccation tube filled with desiccated and PH-free sodium sulfate, and then a filtering calcium chloride tube containing calcined and purified aluminum oxide. Impurities were trapped in this tube, including polar hydrocarbons. The PH were extracted in the Drexel flask with 40 ml of OSCh brand carbon tetrachloride (CCl_4). The air stream containing nonpolar petroleum hydrocarbons was sprayed on the layer of carbon tetrachloride and subsequently passed into solution. Complete extraction was obtained by passing the air stream through a second Drexel flask also filled with 40 ml of CCl_4. After sampling and CCl_4 extraction had ended, the hydrocarbon-saturated petroleum from the air was poured from the two Drexel flasks into one flask for extracts. Standard solutions were prepared, calibration curves were plotted, and the CCl_4 extracts were analyzed using the method for determination of petroleum products in sea water (Handbook of Methods ... 1977).

Half of the water sampled with the grid sampler in the SML and with a vessel in the sub-superficial layer was filtered through SYNPOR No. 2 membrane filter with pore size of 2.5 microns and total diameter of 65 mm for studying the suspended and dissolved PH in the SML. We assumed that filtration through a filter with this pore size would trap most of the suspended substances, both living and dead. The concentration of dissolved PH was determined based on the difference between the total concentration in the surface microlayer and in the suspended form.

In taking samples from the 0-1.0 m subsurface layer, water from the surface microlayer directly in contact with the atmosphere was prevented from entering the bottle. The sample of water from this layer was taken in a heavy-based 5-liter bottle which was sealed with a stopper before it was submerged; the bottle was lowered and then opened.

The extracts of the water samples taken from the subsurface layer, the SML and also the filters with adsorbed suspended substances from the SML were prepared for determination of PH immediately after sampling ended based on practical recommendations (Methods ... 1977). The extracts were analyzed on the ship 1-1.5 hours after extraction began; the concentrations of PH in the extracts of the samples of water and air and on the filters were measured by a model OIL-102 (Yanagimoto, Japan) IR spectrometer. This device allows evaluating the total concentration of petroleum hydrocarbons without identifying their constituent components.

POLLUTION FROM PETROLEUM HYDROCARBONS (PH)

The analysis of the numerous data (Table 1) indicated that the surface microlayer is a powerful concentrator of petroleum hydrocarbons and also of synthetic surfactants and organochlorine pesticides. The concentration of PH in the SML was generally one to two orders-of-magnitude higher than in the subsurface layer. The high concentration of PH in the SML is due to their physical and chemical properties, primarily the slightly lower specific gravity in comparison to the specific gravity of the sea water and their insignificant solubility. The fact that the sources and channels of PH entry in sea water gravitate to the surface of the sea and that their entry into the marine environment is apparently sufficiently regular so that the run-off of PH from the SML is constantly compensated (in the atmosphere by evaporation of the light fractions and with light spray in the water in dissolution or discharge of suspended lumps) plays a significant role.

The range of changes in the concentrations of PH in the SML is rather broad: from tenths of mgl^{-1} to 15 mgl^{-1} with average concentrations from 1 mgl^{-1} to 5.5 mgl^{-1}. To describe the size of these concentrations it is sufficient to recall that the value for the maximum admissible concentration (MAC) for PH in reservoirs used as fisheries in the Soviet Union is a total of only 0.05 mgl^{-1}.

The second conclusion which can be drawn from the analysis of the data is that there is a decrease in total concentration of PH in the SML and a simultaneous absolute and relative increase in the suspended PH and a decrease in the dissolved PH fraction (Table 2) as the distance increases from the shore and the shelf where the primary sources and input channels of PH are located.

TABLE 1. FEATURES OF PETROLEUM HYDROCARBONS IN THE SURFACE MICROLAYER AND IN THE 1-METER LEVEL IN DIFFERENT AREAS OF THE NORTHERN ATLANTIC

Level	No. of Determinations	Range of Changes in Concentrations mgl^{-1}	Mean Concentration mgl^{-1}	Mean Square Deviation mgl^{-1}
20 passages "Tradewind" in the Canary current region (August 1976)				
SML	38	1.2-15.0	5.49	0.04
1 m	38	0.0-0.20	0.06	0.001
15 passages "Monsoon" in the Northern tradewind current region (Winter 1975)				
SML	121	0.2-2.60	1.40	0.08
1 m	121	0.0-0.15	0.03	0.001
23 passages "Monsoon" in the northeastern region of the North Atlantic (Winter 1977)				
SML	99	0.20-2.87	0.95	0.06
1 m	99	0.00-0.05	0.001	0.0001

This is due chiefly to evaporation of PH with low molecular weights in the atmosphere and conversion of part of the dissolved fraction into a suspended fraction in the water.

Table 2 indicates that the mean concentration of the soluble PH fraction changed from 2.7 mg/liter at the shelf to 0.4 mg/liter away from shore, and the mean relative change was 58% (from 83% to 25%). This was due to an absolute decrease in the total concentration of PH in the SML in the open ocean. The mean relative decrease in the total concentration of PH in the SML away from the shelf in the open ocean was approximately 40%. The mean total concentration of PH on the shelf was actually 3.1 mg/liter and only 1.7 mg/liter at the boundaries of the sections in the open sea (data from the last column in the second row in Table 2). The mean concentration of the suspended PH fraction simultaneously increased from 0.4 to 1.3 mg/liter due to conversion of part of the dissolved PH fraction into the suspended fraction, and the mean relative concentration of the suspended PH fraction increased from 17% at the shelf to 75% in the open sea.

As the distance from the shelf increased, the decrease in the concentration of the dissolved PH fraction in the SML caused a decrease in the total concentration and simultaneous increase in the concentration of the suspended fraction.

TABLE 2. CHANGE IN CONCENTRATION OF SUSPENDED AND DISSOLVED FRACTION OF PETROLEUM HYDROCARBONS IN THE SURFACE MICROLAYER IN THE DIRECTION OF THE SHELF ZONE

Section (Conditional Numbering)*	Concentration of suspended (top line) and dissolved (bottom line) fractions of petroleum hydrocarbons	
	mg/liter	% of Total Concentration
1	0.2-2.0 8.0-1.0	2-67 98-33
2	0.4-1.5 3.0-1.0	18-60 82-40
3	0.6-1.2 1.6-0.2	27-86 73-14
4	0.2-1.4 1.2-0.0	14-100 86-0
5	1.0-1.6 1.8-0.6	36-73 64-27
6	0.0-0.4 1.6-0.2	0-67 100-33
7	0.2-0.6 0.8-0.1	20-86 80-14
Mean	0.4-1.3 2.7-0.4	17-75 83-25

* Interpretation of the sections:

No. 1 - from the Straits of Gibraltar to the Cape Verde Islands.
No. 2 - from the Africa shelf 25° north lat. to 40° west long.
No. 3 - from the South America shelf 32° north lat. to 45° west long.
No. 4 - from the Scotland shelf to the Greenland shelf
No. 5 - from the Island shelf to a point 60° north lat. and 25° west long.
No. 6 - from the Norway shelf to the Faeroe Islands
No. 7 - from the Straits of Gibraltar to a point 36° north lat. and 35° west long.

It was suggested above that the decrease in the concentration of the dissolved PH fraction in the SML was caused by their partial evaporation in the atmosphere, dissolution in the underlying layers during dispersion and transfer of part of the dissolved fraction into the suspended fraction in the SML; the latter is supported by the data in Table 2 to a significant degree. For this reason, it was extremely important to determine if there are qualitative or even quantitative correlations between the concentration of PH in the SML on the one hand, and their concentration in the adjacent layer of the atmosphere and the subsurface layer of water, respectively, on the other hand. It is necessary to remember that the concentrations of PH in the water are 1 to 2 orders-of-magnitude lower, and the concentrations in the air are 2 orders-of-magnitude lower than in the SML.

The analysis of the observations conducted in parallel on the SML at a depth of under 1 m and in the air at a height of 0.5 m showed that there is no correlation between the total concentration of PH in the SML, the air and the subsurface layer. This could be due to the different fractional composition of the PH in these media (predominance of the suspended fraction in the water and volatile components in the air), the different additional routes by which they can enter these media (advection of PH in water, in addition to their entry to the SML), etc. In addition, a detailed analysis resulted in the conclusion that there are definite direct correlations (Figure 1) between the concentration of the dissolved PH fraction and the concentration of PH in the atmospheric layer adjacent to the water. These correlations are not identical for different areas of the ocean, however. It is characteristic that the tangent of the angle of inclination of these relations decreases as the distance from the shelf increases and has a smaller value in the open ocean, in the area of oceanic weather station "C" with coordinates of 52°45'N lat. and 35°30'W long. This correlation essentially loses its significance in the open part of the ocean.

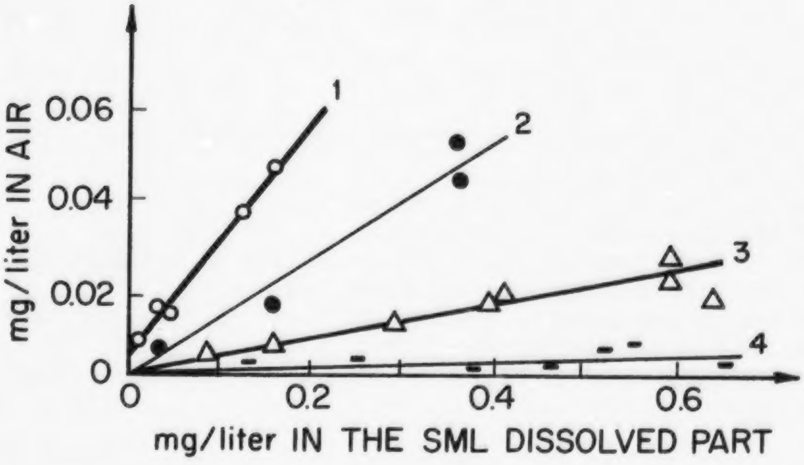


Figure 1. Graph of the correlation between the concentrations of petroleum hydrocarbons in the atmospheric layer adjacent to the water and the dissolved petroleum hydrocarbons in the surface microlayer of the water based on observations: I - northeast section of the Atlantic (near Ireland); II - North Sea; III southern Iceland shelf; IV - oceanic weather station "C".

The reason for the decrease in the degree of the effects of the PH concentrations in the SML on their concentration in the atmospheric layer adjacent to the water as the distance between the observation area and the shore increases is based on the fact that freshly discharged PH in which volatile fractions predominate usually predominate in the coastal regions, while heavy, non-volatile fractions predominate away from coastal regions.

It is believed that the difference in the correlation is caused by other factors: anemobaric conditions, moisture in the air which increases the partial pressure of the PH in the air, the difference in the PH in the SML and the air, turbulent conditions in the boundary layer, etc.

Three circumstances have been determined: A significantly lower concentration of PH in the air than in the SML, a decrease in the soluble PH fraction in the SML as the distance from the shore increases, and the presence of a correlation between the concentration of PH in the air and the concentration of the soluble PH fraction in the SML have resulted in a quantitative evaluation of the process in which part of the PH is transferred from the SML to the atmospheric layer adjacent to the water. This does not mean that the process cannot proceed in the opposite direction, e.g., intake of PH from the atmosphere with precipitation.

In concluding this section, we note that establishing the fact of the high concentration and universal high concentration of PH in the SML will result in an orientational quantitative evaluation of the concentration of PH on the surface of the Pacific Ocean. We hypothesize that the mean concentration of PH in the SML in Pacific Ocean waters will fluctuate from 2 to 3 mg/liter. Of course, it could be significantly lower in certain areas. However, this hypothesis is based on the presence of petroleum films in many areas containing a concentration of PH which is many times higher than the concentration indicated above. Based on these petroleum films, we hypothesize that the average thickness of the SML varies from 250-750 μm. On the basis of these hypotheses, the concentrations of PH in the SML in all water areas in the Pacific Ocean could be estimated at 0.54-2.20 million tons.

Approximately 5.5 million tons of petroleum and petroleum products enter the Pacific Ocean each year (Goldberg 1976) as a result of anthropogenic activity. The value for PH contained in the SML calculated above represents 10-35% of the annual discharge into oceans and seas. Of course, it is difficult to give preference to any extreme value for the relative estimation of the concentration of PH on the surface of the Pacific Ocean. In any case, the amount of PH in the SML seems to be very large. This circumstance emphasizes the role of the boundary surfaces in the powerful concentration of PH and indicates the need to study their concentration in the other two boundary surfaces: in the bottom sediment and in the density discontinuity layers. This in turn would facilitate the development of strategies for monitoring petroleum pollution in the marine environment. Knowing the amount of PH contained in the SML (M = 540,000-2,200,000 tons) and their rate of entry (γ = 5.5 million tons a year) would make it possible to calculate the estimated time (T) the PH remain in the SML. We advance two hypotheses: First, all or a large part of the PH which enter the sea necessarily pass into a stage of concentration in the SML. The time they remain in SML can only be correctly calculated in this case. If we consider the phase of petroleum film formation, this hypothesis is essentially confirmed. Second, the concentration of PH in the SML does not change or changes very little with time (a number of years). In this case, we could use a simple ratio for calculating the time the PH remain in the SML: $T = M:\gamma$. The second hypothesis is apparently not always plausible: One to two decades ago, the concentration of PH in the SML primarily tended to increase; now, however, the concentration apparently tends to decrease due to the water conservation measures instituted in many countries. The time that PH remain in the SML can also be calculated for the case where the mean concentration of PH changes in time, but the formula is more complex. However, the quantitative features of the dynamics involved in the change in PH are not known at the present time.

The time that PH remain in the SML could be from 1-5 months based on the initial parameters and hypotheses indicated above. The time calculated in this way is not only in agreement with respect to the order-of-magnitude, but is also close to the value of the experimentally determined half-life of petroleum dissolved and dispersed in water (Simonov *et al*. 1978). The half-life of petroleum in sea water at temperatures of 0-10°C is equal to approximately 1.5 months; as the temperature increases, the value decreases: At 18-20°C, the half-life is equal to approximately 20 days, and at 25-30°C--7 days. We again emphasize that the half-life of PH coincides with the time PH remain in water and that the time segment which is sufficiently close to the total decomposition time will be greater than the values calculated for T.

Based on the comparisons of the values for T and τ, we can assume that only approximately half of the PH found in the SML can be suspended and dissolved in the water at low water temperatures, and again become concentrated in the density discontinuity layers or in the bottom sediment, while the very volatile components enter the atmosphere.

The other half of the PH can decompose in the SML. With high water temperatures, a significant part of the PH, except for the very volatile components, can decompose directly in the SML. Both cases (together with turbulent diffusion) would completely explain the decrease in the concentration of PH in the SML as the distance from a shelf (pollution foci) to the open ocean increases, and would also indicate the possibility of significant accumulation of PH at mean and high latitudes and, on the contrary, the low accumulation in equatorial, tropical, and subtropical zones.

Comparision of the values for T and τ also leads to the conclusion that the SML is not only a powerful PH concentrator, but is also a PH filter, since a significant part of the PH can decompose in this layer, protecting the mass of the ocean from intensive pollution. This conclusion is extremely important in correctly calculating the basic components in the accounts of PH pollution of the marine environment reflecting the dynamics of the levels of pollution. Up to now, the concentration of a significant part of PH in the comparatively small volume of Pacific Ocean water contained in the SML, consisting of less than 1% of the total volume of water, and their degradation in the SML have not been calculated in the expenditure part of the accounts. The equation for case. If we consider the phase of petroleum film formation, this hypothesis is essentially confirmed. Second, the concentration of PH in the SML does not change or changes very little with time (a number of years). In this case, we could use a simple ratio for calculating the time the PH remain in the SML: $T = M:\gamma$. The second hypothesis is apparently not always plausible: One to two decades ago, the concentration of PH in the SML primarily tended to increase; now, however, the concentration apparently tends to decrease due to the water conservation measures instituted in many countries. The time that PH remain in the SML can also be calculated for the case where the mean concentration of PH changes in time, but the formula is more complex. However, the quantitative features of the dynamics involved in the change in PH are not known at the present time.

The time that PH remain in the SML could be from 1-5 months based on the initial parameters and hypotheses indicated above. The time calculated in this way is not only in agreement with respect to the order-of-magnitude, but is also close to the value of the experimentally determined half-life of petroleum dissolved and dispersed in water (Simonov et al. 1978). The half-life of petroleum in sea water at temperatures of 0-10°C is equal to approximately 1.5 months; as the temperature increases, the value decreases: At 18-20°C, the half-life is equal to approximately 20 days, and at 25-30°C--7 days. We again emphasize that the half-life of PH coincides with the time PH remain in water and that the time segment which is sufficiently close to the total decomposition time will be greater than the values calculated for T.

Based on the comparisons of the values for T and τ, we can assume that only approximately half of the PH found in the SML can be suspended and dissolved in the water at low water temperatures, and again become concentrated in the density discontinuity layers or in the bottom sediment, while the very volatile components enter the atmosphere.

The other half of the PH can decompose in the SML. With high water temperatures, a significant part of the PH, except for the very volatile components, can decompose directly in the SML. Both cases (together with turbulent diffusion) would completely explain the decrease in the concentration of PH in the SML as the distance from a shelf (pollution foci) to the open ocean increases, and would also indicate the possibility of significant accumulation of PH at mean and high latitudes and, on the contrary, the low accumulation in equatorial, tropical, and subtropical zones.

Comparision of the values for T and τ also leads to the conclusion that the SML is not only a powerful PH concentrator, but is also a PH filter, since a significant part of the PH can decompose in this layer, protecting the mass of the ocean from intensive pollution. This conclusion is extremely important in correctly calculating the basic components in the accounts of PH pollution of the marine environment reflecting the dynamics of the levels of pollution. Up to now, the concentration of a significant part of PH in the comparatively small volume of Pacific Ocean water contained in the SML, consisting of less than 1% of the total volume of water, and their degradation in the SML have not been calculated in the expenditure part of the accounts. The equation for the accounts (Simonov et al. 1978) should apparently be made more accurate by introducing an additional component--chemical and biochemical decomposition of PH in the SML.

POLLUTION FROM SYNTHETIC SURFACTANTS (SS) AND ORGANOCHLORINE PESTICIDES (OCP)

As in the case of petroleum hydrocarbons, an analysis of the data allows the conclusion that significant quantities of synthetic surfactants, which enter the marine environment with discharge of wastes from the shore, river waters and ships, are concentrated in the surface microlayer. The concentration of SS in the SML could be 1 to 2 orders-of-magnitude higher than in the subsurface layer. This can be seen from the features of SS shown in Table 3. The range of changes in the concentration of SS in the SML reaches 1000 μg/liter with an average value of 820 μg/liter. Similar to the case of PH, the mean concentration of SS is many times higher than the MAC, which is set at 100 μg/liter. In addition, the concentration of SS in the subsurface layer is significantly lower than the MAC.

TABLE 3. CHARACTERISTICS OF SS IN THE SML AND AT THE 1-METER LEVEL IN THE ATLANTIC OCEAN, ON THE NORTHWESTERN COAST OF AFRICA (in g/liter; 20 passages of the TRADEWIND in August 1976)

Level	No. of Determinations	Range of Changes	Mean Concentration	Mean Square Deviation	Interval of Significance
SML	38	180-1250	820	10.1	820±12.4
1 m	38	15-60	34	5.1	34±6.3

The same features are observed in the spatial distribution of SS as for PH: The concentration decreases as the distance from the shore increases. Thus, the concentrations of SS were over 1000 μg/liter in the region from the Straits of Gibraltar to the Canary Islands (longitude 17-18°W), and the maximum reached 1220 μg/liter. The significant concentration of SS in this region is related to the effect of the polluted waters in the Straits of Gibraltar, effluence from the coast of Africa and intensive navigation which was also found for the high concentrations of PH in the SML discussed above.

In moving to the open ocean, the concentration of SS decreases to 200 μg/liter in the region 20-21°W longitude. The decrease in the concentration of SS is basically due to their dynamic dispersion. The concentration in the subsurface layer in going from the Straits of Gibraltar to the open ocean decreases from 50-60 to 20 μg/liter and less.

All of the characteristic features of the concentration of PH in the SML and their spatial distribution also apply to SS; this is not only due to the same sources and channels of PH and SS input, the similarity of some of their physical and chemical properties (lower specific gravity with respect to sea water, low solubility), but also to the fact that the concentration of PH increases due to their emulsification in surface films.

In turning to the characteristics of the concentrations and spatial distribution of organochlorine pesticides, we emphasize that most of them are difficult to dissolve in water, are easily soluble in organic solvents and ethers, and can be adsorbed in marine organisms and suspended substances. In light of what was shown for PH in the SML and since the suspended matter, living and dead organisms are concentrated in the SML and since the atmosphere is the basic means by which OCP enter the marine environment, we can hypothesize that the concentration of OCP in the SML should be high, especially in areas with significant concentrations of PH. The results of studies in 23 passages of the "Monsoon" in the winter of 1977 in the northeastern section of the Atlantic Ocean confirm this hypothesis. The concentration of OCP in the SML is a characteristic feature in comparison with the concentration in the subsurface layer (Table 4).

TABLE 4. MEAN CONCENTRATIONS (top figure) AND RANGE OF VARIABILITY (bottom figure) IN THE CONCENTRATIONS OF PESTICIDES (in ng/liter based on data from 23 voyages of the NISP MONSOON, Winter 1977)

Level	DDT	DDE	DDD	τ-HCCH
SML	33.7 0.2-132	7.6 0.0-51.5	7.8 0.0-32.0	26.0 0.0-148
1 m	2.04 0-17.6	0.53 0-2.0	1.22 0-4.4	0.89 0-2.0

Judging by the mean concentrations, the concentration of all pesticides in the SML is an order-or-magnitude higher than in the subsurface layer. The highest concentrations of DDT were found over the Ireland shelf, where they reached 80 ng/liter (Figure 2a), while they fluctuated from 2-4 ng/liter in the subsurface layer. As a function of the distance from the North Altantic current streams, the concentration of DDT sharply decreased and was approximately 10 mg/liter in the North Atlantic water mass. The decrease in the concentrations of DDT is not only due to their dynamic dispersion in dissemination from islands. It is also related to conversion of part of the DDT into metabolites. Figure 2b shows that the spatial distribution of the total metabolites (DDD+DDE) is inverse to the distribution of DDD. The total concentrations of metabolites increases with distance from islands, and attains maximum values in the waters of the North Atlantic current and the southern approaches to the Danish Straits.

We note that although approximately 69% of the total DDT, DDD, and DDE is represented in the mean concentration of DDT in the SML, it decreases to 40-50% in the waters of the North Atlantic current.

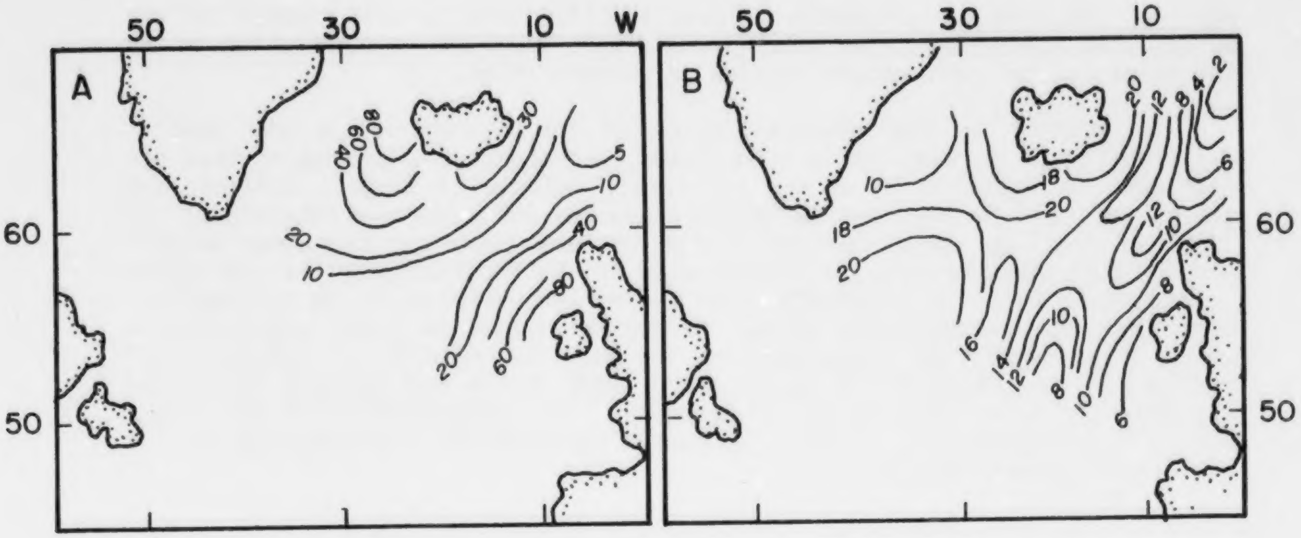


Figure 2. Spatial distribution of DDT (a) and total DDD and DDE (b) in the northeastern part of the Atlantic Ocean, in ng/liter, based on observations in the winter of 1977.

The fact that the maximum values (20-50 mg/liter) are observed in the southern approaches to the Danish Straits is characteristic in the spatial distribution of ϒ-HCCH in the SML; the concentrations of ϒ-HCCH in the SML in the region of the Faeroe Island shelf and the Faeroe-Scotland straits falls to zero values. The concentration of ϒ-HCCH in the subsurface changed insignificantly (from 0 to 2 mg/liter).

Certain general features thus exist in the concentration of PH, SS, and OCP in the SML and in their spatial distribution. However, in contrast with the other pollutants, the concentration of OCP in the SML can decrease to zero in moving away from shelves.

CONCLUSIONS

1. Based on multiple two-year observations by the GOIN NISP in the North Atlantic:

   1.1. A significant concentration of petroleum hydrocarbons, synthetic surfactants and chlorinated hydrocarbons was found in the surface microlayer of water; these substances entered the Pacific Ocean in different ways;

   1.2. It was found that the concentrations of these substances in the surface microlayer was 1 to 2 orders-of-magnitude higher than the concentrations in the subsurface layer and the atmospheric layer adjacent to the water;

1.3. The spatial changes in the concentrations of chemical pollutants were investigated in the surface microlayer of water under the effect of external physical-chemical factors and current systems; it was found that they generally decreased as the distance from the mainland or shelf zones increased, but still remained comparatively high except for chlorinated hydrocarbons, whose concentration decreased to traces. The qualitative composition of the pollutants simultaneously changed as the distance from the shore increased: The suspended portion absolutely and relatively increased and the dissolved portion of the petroleum hydrocarbons decreased; the absolute and relative fractions of DDE and DDD (DDT metabolites) also increased and the DDT fraction decreased;

1.4. It was shown that high concentrations of petroleum hydrocarbons and synthetic surfactants in the surface microlayer of the water were characteristic of all water areas in the North Atlantic, and high concentrations of chlorinated hydrocarbons were characteristic of significant expanses in the shelf zones of the North Atlantic.

2. On this basis, we drew a conclusion concerning the global nature of the appearance of stable, high concentrations of chemical pollutants in the surface microlayer of water in the Pacific Ocean and the global nature of the disturbances in the naturally combined physical-chemical features of the surface microlayer of the Pacific Ocean under the effect of pollution.

These conclusions indicate the need for the following:

2.1. Organizing systematic observations of the pollution in the surface microlayer of water both in oceans and in the seas in the Soviet Union; this would allow more precise calculation of the pollutants contained in this layer;

2.2. Calculating the amounts of pollutants contained in and decomposing in the surface microlayer (primarily petroleum hydrocarbons) in calculating the amounts in seas and predicting the level of pollution in seas using the balance method.

REFERENCES

Garret, W. D. 1972. Impact of natural and surface film on the ocean. Nobel Symposium 20. Almqvist and Wiknell, Stockholm, pp. 75-81.

Goldberg, E. D. 1976. The health of the oceans. UNESCO Press, pp. 117-128.

Handbook of methods for the chemical analysis of marine waters. 1977. Gidrometeoizdat, pp. 118-127, 131-136, 145-155.

Oradovskiy, S. G., A. I. Simonov, and A. A. Yushchak. 1975. Study of the distribution of chemical pollutants in the Gulfstream zone and their effect on primary production of oceanic waters. Meteorologiya i gidrologiya, 2:48-58.

Simonov, A. I., S. G. Oradovskiy, and A. A. Yushchak. 1974. The current state of pollution in the North Atlantic. Meteorologiya i gidrologiya, 3:61-69.

Simonov, A. I., N. A. Afanas'yeva, T. A. Bakum, and B. M. Zatuchnaya. 1978. Self-purification processes in sea water with respect to chemical pollutants. Trudy GOINa, 128:96-104.

STUDIES ON THE BIOLOGICAL TRANSPORT OF MATERIALS FROM SURFACE TO DEEP OCEAN WATERS: I. FLUXES OF CARBON, NITROGEN AND PHOSPHORUS II. FLUXES OF TRACE ELEMENTS

by

G. A. Knauer and J. H. Martin
Moss Landing Marine Laboratories
Moss Landing, California

INTRODUCTION

It has long been recognized that the plankton play an important role in the biogeochemical cycles of various materials. For this reason, a great deal of research has been devoted to the analyses of plankton remains (oozes) on the sea floor (e.g., Arrhenius 1963) and plankton/sediment interaction (Manheim et al. 1972). The plankton living in the surface waters of the oceans have also been analyzed for trace elements (Martin and Knauer 1973; Martin et al. 1976), hydrocarbons (Conover 1971), etc., under the assumption that much of this material will sink from the surface mixed layer and eventually transport associated elements, compounds, etc. to mid-depths and to the bottom. Thus, considerable data exist on the plankton in the surface waters and their remains on the sea floor.

In addition, the types of material carried to various depths by particles are becoming well documented through sinking rate studies; considering fecal pellets alone, for example, there are convincing data with respect to trace metals (Lowman et al. 1971; Boothe and Knauer 1972; Small et al. 1973; Small and Fowler 1973; Benayoun et al. 1974; Bishop et al. 1977; Fowler 1977; Spencer et al. 1978); skeletal structures and frustules (Schrader 1971; Ferrante and Parker 1977; Roth et al. 1975; Honjo 1976); alpha emitters and transuranic elements (Cherry et al. 1975; Bacon et al. 1976; Beasley et al. 1978; Higgo et al. 1977); chlorinated hydrocarbons (Elder and Fowler 1977); petroleum hydrocarbons (Conover 1971).

However, there are few data for the most important measurement needed, i.e., the actual flux of detritus and associated materials as they leave the surface and sink through the water column. The lack of this type of information has hindered our ability to deal with specific problems such as removal of inorganic and organic pollutants from the sea surface, understanding nutritional aspects of the mid-water column and deep-sea benthos, determining the fate of "excess" industrially produced CO_2 and even unraveling the basic biogeochemistry of the world ocean.

During the past two or three years, the means have become available to assess fluxes of materials, not only to the sea floor but also through various portions of the water column (e.g., Wiebe et al. 1976; Soutar et al. 1977; Bishop et al. 1977; Knauer et al. 1979). Recent advances in methodology and instrumentation have also made possible the accurate measurement of elements and compounds at the very low levels at which they exist in sea water (e.g., Boyle and Edmond 1975; Moore and Burton 1976; Boyle et al. 1977; Bruland et al. 1978ab; Bruland et al. 1979). The combination of developments now enables oceanographers to measure amounts added or removed (rates of change) by comparing fluxes at various depth intervals. In other words, we can now study the processes and measure the rates that determine the distribution of elements and/or compounds in the water column.

However, because of the complexity of the vertical transport problem in terms of interacting oceanographic variables (e.g., rates of primary productivity, particulate vs. dissolved components, season, sample depth, and particle interceptor trap design, etc.), we do not feel that isolation of one single topic for discussion (e.g., fluxes of trace elements from surface to deep ocean waters) would present the clearest picture in a dynamic sense. Therefore, we would like to present various aspects of the vertical flux problem that we are actively working on at the present time. This will be done through the use of tables and figures which will be used to illustrate various points. The tables and figures are organized according to related content and will be preceded by a topic phrase or sentence followed by a brief discussion of their relevance.

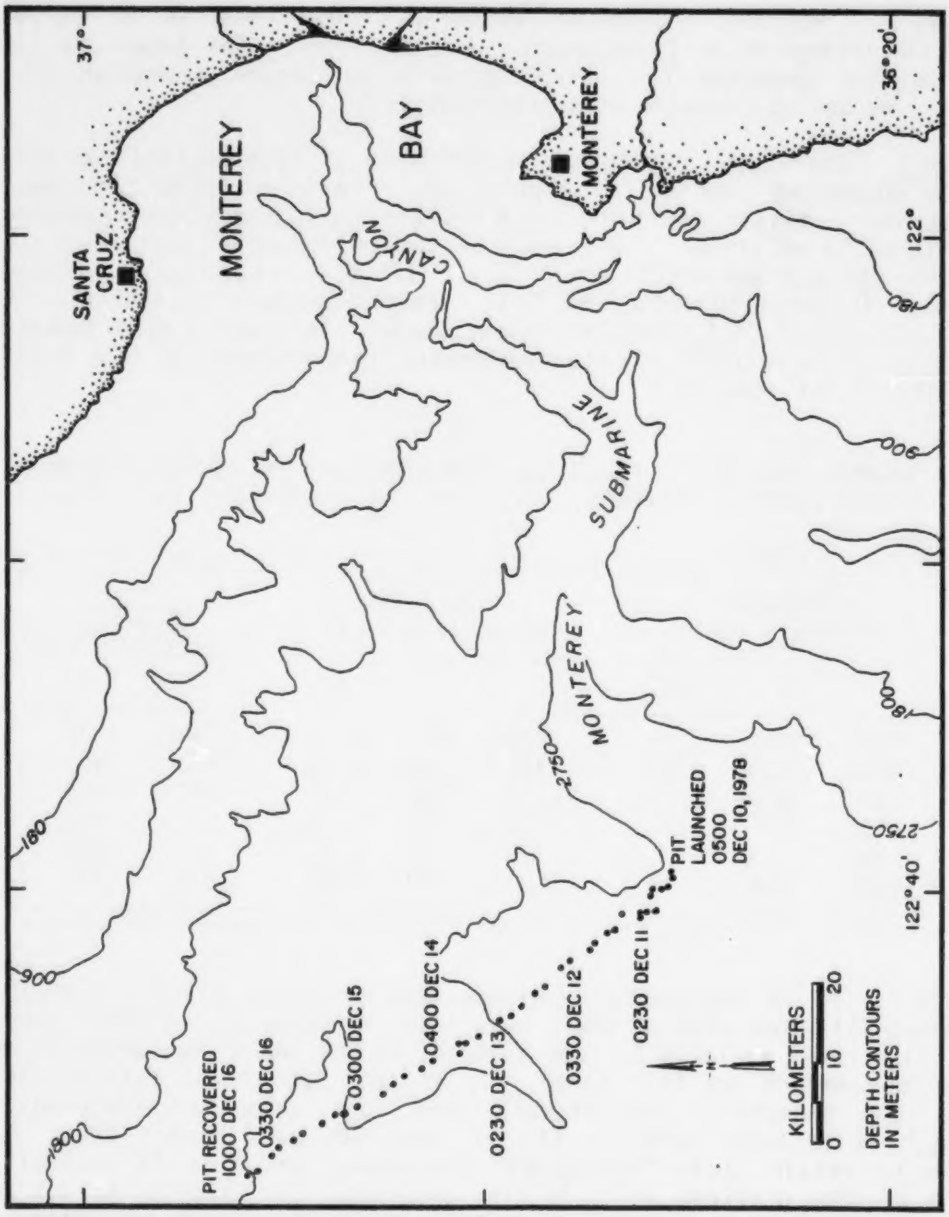
We have included no major discussion in the text at this time, since many of the data here have been recently obtained and many samples are yet to be analyzed. However, even now the data suggest some exciting results and trends, and we are looking forward to feedback and general discussion from our Soviet counterparts engaged in similar research.

METHODS

Many of the methods pertaining to MULTIPIT design and sample processing can be found in Knauer et al. 1979 (Fluxes of particulate carbon, nitrogen, and phosphorus in the upper water column of the Northeast Pacific, Deep Sea Research 26(1A):97-108).

In terms of metal analysis, procedures involving clean techniques such as the use of portable ship-going laboratories, clean-up, etc. can be found in Bruland et al. 1979 (Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel at the nanogram per liter level in sea water, Anal. Chem. Acta, 105:233-245).

Most of the data presented here were obtained using a free-floating MULTIPIT system set approximately 60 km off the Central California coast for 6 days (Figure 1). The MULTIPITs were placed at 35, 65, 150, 500, 750, and 1,500 meters. The vessel used was the R/V WECOMA, December 1978.



SANTA CRUZ
MONTEREY
MONTEREY BAY
MONTEREY SUBMARINE CANYON
37°
36°20'
122°
122°40'
180
900
1800
2750
PIT LAUNCHED 0500 DEC 10, 1978
0230 DEC 11
0330 DEC 12
0230 DEC 13
0400 DEC 14
0300 DEC 15
0330 DEC 16
PIT RECOVERED 1000 DEC 16
N
KILOMETERS
0 10 20
DEPTH CONTOURS IN METERS

Figure 1.

RESULTS

I. Mechanics

Figure 2. MULTIPIT collector assembly. The cross is of polyvinyl chloride consisting of 8-12 individual acrylic tubes. The tubes are filled with a density gradient (ε = 1.07 g/cm^{-3}) to insure retention of the particles. We use 5% formalin as a preservative.

Table 1. There are zooplankton in the area of each MULTIPIT as well as vertically migrating zooplankton populations. We have found that some of these organisms actively swim into the MULTIPITs and die upon encountering the gradient-formalin solution. These must be removed by hand (dissecting scope) or considerable contamination can result. Table 1 illustrates this point. For example, in the 1,500 meter MULTIPIT, removed swimmers account for 4,893, 624, and 1.16 µg of C, N, and Zn, respectively. This would contribute 310, 330, and 30 percent more of these elements respectively to the MULTIPIT particulates if not removed.

TABLE 1. SWIMMER EFFECTS: POTENTIAL CONTRIBUTION OF REMOVED SWIMMERS TO TOTAL CARBON, NITROGEN, AND ZINC IN MULTIPITS (from Knauer and Martin)

MULTIPIT Depth (m)	Removed Swimmers (µg)			MULTIPIT Particulates (µg)			$\frac{\text{Swimmers}}{\text{Particulates}} \times 100$		
	C	N	Zn	C	N	Zn	C	N	Zn
35	5485	1123	1.21	7328	1474	7.28	75	76	17
65	5257	939	0.97	3384	525	5.96	155	120	16
150	5370	1067	0.78	1819	258	3.81	295	414	20
500	1059	230	0.70	493	67	1.87	215	340	37
750	1546	245		473	54	1.64	327	455	--
1500	4893	624	1.16	1578	189	3.82	310	330	30

Table 2. It is reasonable to assume that particles sinking through the water column will lose some of their associated contents during their descent. This is illustrated in Table 2. For example, at the end of two weeks (some of our sampling periods are this long), 77, 89, and 97% of the initial carbon, nitrogen, and phosphorus respectively were lost under the "decomposition treatment," while with formalin 32, 40, and 58% were lost. Thus, it is important to retain these "dissolved" compounds, and this is accomplished using the density gradient which is also analyzed. For example, to get total carbon, you must analyze the carbon in both the "particulate" and "dissolved" fractions.

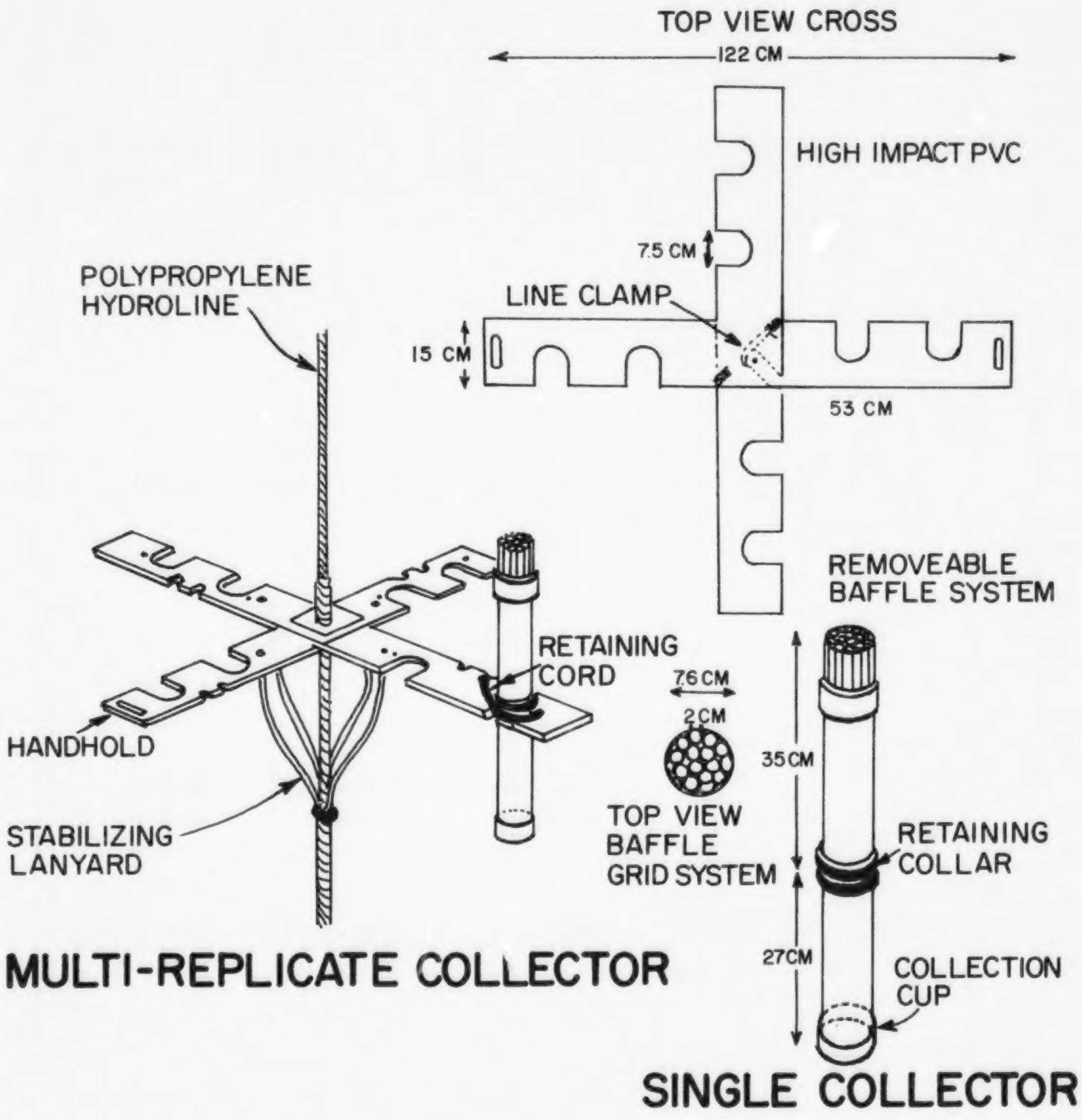

TOP VIEW CROSS
122 CM
HIGH IMPACT PVC
7.5 CM
LINE CLAMP
15 CM
53 CM
REMOVEABLE BAFFLE SYSTEM
POLYPROPYLENE HYDROLINE
RETAINING CORD
HANDHOLD
STABILIZING LANYARD
7.6 CM
2 CM
TOP VIEW BAFFLE GRID SYSTEM
35 CM
RETAINING COLLAR
27 CM
COLLECTION CUP
MULTI-REPLICATE COLLECTOR
SINGLE COLLECTOR

Figure 2.

TABLE 2. DECOMPOSITION: MIXED PLANKTON TOW COLLECTED AND PLACED IN METAL STRIPPED SEA WATER AT 35% SALT. DECOMPOSITION ALLOWED TO PROCEED FOR TWO WEEKS AT ROOM TEMPERATURE (from Knauer and Martin)

Treatment	Initial Wet Wt. (g)	Final Wet Wt. (g)	% Wt. Loss	Total CNP (mg)			% Loss of Initial		
				C	N	P	C	N	P
Initial									
1	1.57			51	13	1.1			
2	1.78			57	14	1.1			
3	1.64			57	14	0.9			
4	1.70			37	9	0.9			
$\bar{X}$	(1.67)			(51)	(13)	(1.0)			
Decomposition									
1	1.62	0.55		11	1.3	0.04			
2	1.70	0.56		11	1.3	0.03			
3	1.67	0.57		11	1.4	0.03			
4	1.68	0.61		13	1.6	0.03			
x	(1.67)	(0.57)	(66)	(11.5)	(1.4)	(0.03)	(77)	(89)	(97)
5% Formalin									
1	1.48	1.08		34	8	0.43			
2	1.70	1.10		33	7	0.41			
3	1.65	1.14		40	9	0.43			
4	1.63	1.09		31	7	0.42			
x	(1.62)	(1.10)	(32)	(34.5)	(7.8)	(0.42)	(32)	(40)	(58)
Azide (10 g/1)									
1	1.52	0.67		14	3.0	0.07			
2	1.66	0.75		18	5.2	0.10			
3	1.67	0.66		14	3.8	0.07			
4	1.78	0.83		19	5.3	0.10			
x	(1.66)	(0.73)	(56)	(16)	(4.3)	(0.09)	(69)	(67)	(91)

Table 3. This table illustrates the above effect with actual MULTIPIT field samples for cadmium, copper, manganese, and PO_4^{-3}. For example, only 17% of the Cd is retained on the particles, while 83% is lost to the density gradient. Copper, on the other hand, is strongly bound to the particulate fraction.

TABLE 3. PARTICULATE/DISSOLVED: SEPARATE ANALYSIS OF MULTIPIT PARTICULATE AND DISSOLVED FRACTIONS (from Knauer and Martin)

MULTIPIT Depth (m)	Cd (ng)		Cu (ng)		Mn (ng)		PO_4 (μg)	
	Part.	Diss.	Part.	Diss.	Part.	Diss.	Part.	Diss.
35	43	248	274	<22	3,760	1,633	124	196
65	25	139	324	<22	5,377	1,329	51	82
150	11	60	314	<22	6,020	743	26	54
500	4	19	261	<22	3,926	192	7.3	10.5
750	3	14	230	<22	3,285	232	5.5	12.2
1,500	5	18	410	<22	4,855	214	12.7	33

II. Fluxes of Carbon, Nitrogen, and Phosphorus

One of the major questions we are concerned with is: Of the total organic material produced in the euphotic zone by the phytoplankton (primary productivity), what fraction reaches various depths in the water column? This is important in terms of biogeochemical cycles as well as pollutant transfer. Data pertaining to this question are presented in Tables 4 and 5.

Table 4. These data represent the total amount of carbon fixed throughout the euphotic zone over the duration of the MULTIPIT set (i.e., 6 days). The method used for primary productivity measurements was a new metal-free technique developed by us which can be discussed at the Symposium. It is obvious from these data that most of the carbon fixed is recycled in the upper 150 meters. For example, of the total carbon fixed (i.e., 4,105 mg C m^{-2}/6 days), 956 mg m^{-2} or 23% reached 65 meters (= the 1% light level or "bottom" of the euphotic zone). This supports earlier assumptions that most of the material produced in the upper layers of the ocean is regenerated in the upper levels of the ocean (Votinez 1953; Menzel 1974). Note that the percentage appears to increase at 1,500 meters. We think this is an example of the ladder effect suggested by Vinogradov (1961) and can be discussed in more detail at the Symposium.

Table 5. This table presents the amount of C and N collected in each MULTIPIT over the 6-day set. Notice the rapid decrease culminating at the oxygen minimum zone (750 m). Also notice the increase of C and N at 1,500 m (ladder effect?). In terms of C/N ratios, the obtained values seem reasonable. The values 6.0 and 6.9 found over the euphotic zone (0-65 m) were not unexpected since most of the C and N is ultimately plankton-derived and should yield values close to the Redfield *et al*. ratio of 6.6 (Redfield *et al*. 1963).

TABLE 4. CARBON LEAVING THE EUPHOTIC ZONE AS PERCENT OF CARBON (PRIMARY PRODUCTIVITY) FIXED OVER THE 6-DAY MULTIPIT SET (R/V WECOMA, December 1978; from Knauer and Martin)

Date	Integrated Primary Productivity mg C m^{-2} day^{-1}	Depth (m)	Total Average Carbon Collected in MULTIPIT mg m^{-2}	As % of Primary Productivity
1978				
10 December	780	65	956	23
11 December	810	150	530	13
12 December	695	500	107	2.6
13 December	690	700	97	2.3
14 December	650	1,500	290	7.1
15 December	480			
	Σ 4,105 (mg C m^{-2}/6 days)			

TABLE 5. PARTICULATE CARBON AND NITROGEN AND C/N RATIOS / MULTIPIT (R/V WECOMA, December 1978; from Knauer and Martin)

Depth (m)	Carbon (mg)	$\bar{x}$	Nitrogen (µg)	$\bar{x}$	C/N (by Atoms)	$\bar{x}$
35	7,880	7,330	1,590	1,430	5.8	6.0
	6,775		1,270		6.2	
65	3,745	3,450	600	586	7.3	6.9
	3,152		570		6.5	
150	2,080	1,790	315	270	7.7	7.7
	1,500		230		7.6	
500	420	500	58	72	8.5	8.2
	580		86		7.8	
750	380	480	49	57	9.1	9.7
	575		65		10.3	
1,500	1,145	1,810	130	223	10.3	9.7
	2,475		316		9.1	

106C:16N from Redfield _et_ _al_. (1963) = 6.6

Increases in this ratio with depth are also plausible, since the more nutritious nitrogenous substances (N ≡ protein) are being preferentially used while the more refractory carbon is left behind (Knauer and Ayers 1977).

III. The Rain of Detritus

In 1888, Agassiz proposed that "... deep-sea organisms are nourished by a 'rain' of organic detritus from overlying surface waters." We are also examining this question with our MULTIPITs. The data presented in this section reflect this.

Figure 3. Shows the decrease in material collected in sextuplicate. Again, it would seem that most of the material produced at the surface is regenerated above the oxygen minimum. However, there appears to be an increase in total weight collected at 1,500 m. That this is not an artifact is reinforced in the data presented in Table 6.

Table 6. We have been examining and analyzing the MULTIPIT material for exoskeletons, chitin, fecal pellets, etc. These data represent the major size classes of fecal pellets found in our collectors over the 1,500 meter sampling depth. Notice the general decrease in the cylindrical size class. However, the 0.05 and 0.10 mm size class increases at 1,500 meters. We feel this may be an example of repackaging.

TABLE 6. FECAL PELLET DATA, NORTHEAST PACIFIC (WECOMA, December 1978; from Urrere, Knauer, and Martin 1979, in preparation)

	Size Class (mm)									
	Elliptical				Cylindrical					
Depth (m)	0.05	0.10	0.15	0.25	0.05	0.10	0.15	Round	Coiled	Total Flux m^{-2} day^{-1}
35	124	341	230	11	5,013	902	29	725	281	
	181	401	269	11	4,871	1,259	35	674	251	322,660
65	132	116	125	7	4,188	733	20	472	119	
	159	133	109	5	3,831	847	36	395	107	238,440
150	313	80	8	1	3,737	636	28	359	62	
	219	105	16	4	3,127	724	30	289	54	215,990
500	154	140	7	4	299	123	7	78	7	
	175	131	16	2	363	141	8	70	4	35,722
750	540	388	10	7	193	37	13	58	3	
	481	364	10	4	229	33	3	41	3	49,966
1,500	790	759	77	3	135	42	10	76	0	
	864	767	63	5	198	64	13	63	0	84,614

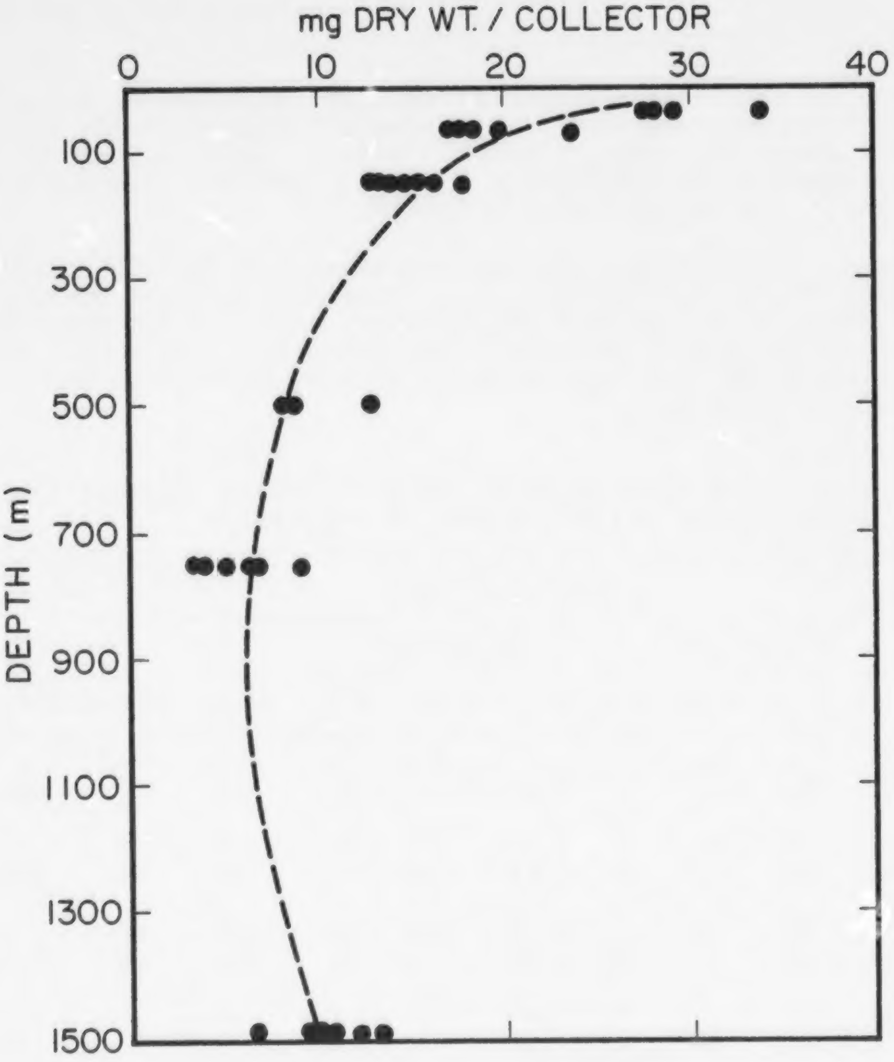

mg DRY WT. / COLLECTOR
0
10
20
30
40
DEPTH (m)
100
300
500
700
900
1100
1300
1500

Figure 3.

Figure 4. This is a plot of ln fecal pellet number (0.10 mm) with depth. There appears to be a good fit to an exponential curve to 750 meters, although this size class at the 1,500 meter depth falls off the line. This may be the result of the ladder effect (see below, Table 7).

Table 7. Analysis of the 0.10 mm size class reveals that the primary food items in these pellets were *Pseudoeunotia doliolus* and *Coscinodiscus* fragments. In general, all pellets from this size class contained the same quantity of these cells except for the pellets analyzed at 750 m. This suggests that some of the organisms from 1,500 meters are migrating up through the oxygen minimum zone to feed.

TABLE 7. DOMINANT FOOD ITEMS IN THE 0.10 mm DIAMETER CYLINDRICAL SIZE CLASS (from Knauer and Martin)

	X Number/Depth (m)					
Food Item	35	65	150	500	750	1,500
Pseudoeunotia doliolus	13	9	17	10	2	12
Coscinodiscus fragments	14	10	8	7	2	9
Prorocentrum sp.	2	1	0	1	0	0.1
Nitzschia-navicula (?)	2	0	0.1	0	1	2
Nitzschia sp.	1	0.1	0	0.1	0	0.4
Dinoflagellate parts	1.5	0.6	0.2	0	0	0
Zooplankton parts	1	0.8	0.2	0.2	0.2	0.3
(n pellets analyzed)	(14)	(7)	(8)	(7)	(6)	(7)

% Dominant Phytoplankton Species (Partial List)

Schroderella delicatula	19	*Pseudoeunotia doliolus*	1.2
Bacteriastrum sp.	17	Coscinodicus (9 species)	0.4
Nitzschia sp.	10	*Ceratium* sp.	0.02
Skeletonema costatum	12	*Prorocentrum* sp.	0.15
Chaetocerus affinis	7		

IV. Metal Fluxes

We are presently analyzing our MULTIPIT samples for Al, Ag, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. However, we are only in a position to discuss a few of these elements at this time. Three examples (Mn, Pb, and Cd) are presented below.

Figure 5. Shows oxygen distribution (ml O_2/l) and dissolved Mn (ng/l). [Dissolved Mn is defined here as Mn remaining after passing a seawater sample (10-30 l) through acid washed 0.4 μ nucleopore filters.] It can be seen that there is a rapid decrease in dissolved Mn from surface waters (100-200 ng/l) to depth (40-60 ng/l). It is interesting to note that there is a slight maximum in the oxygen minimum zone which may suggest a redissolution of MnO_2.

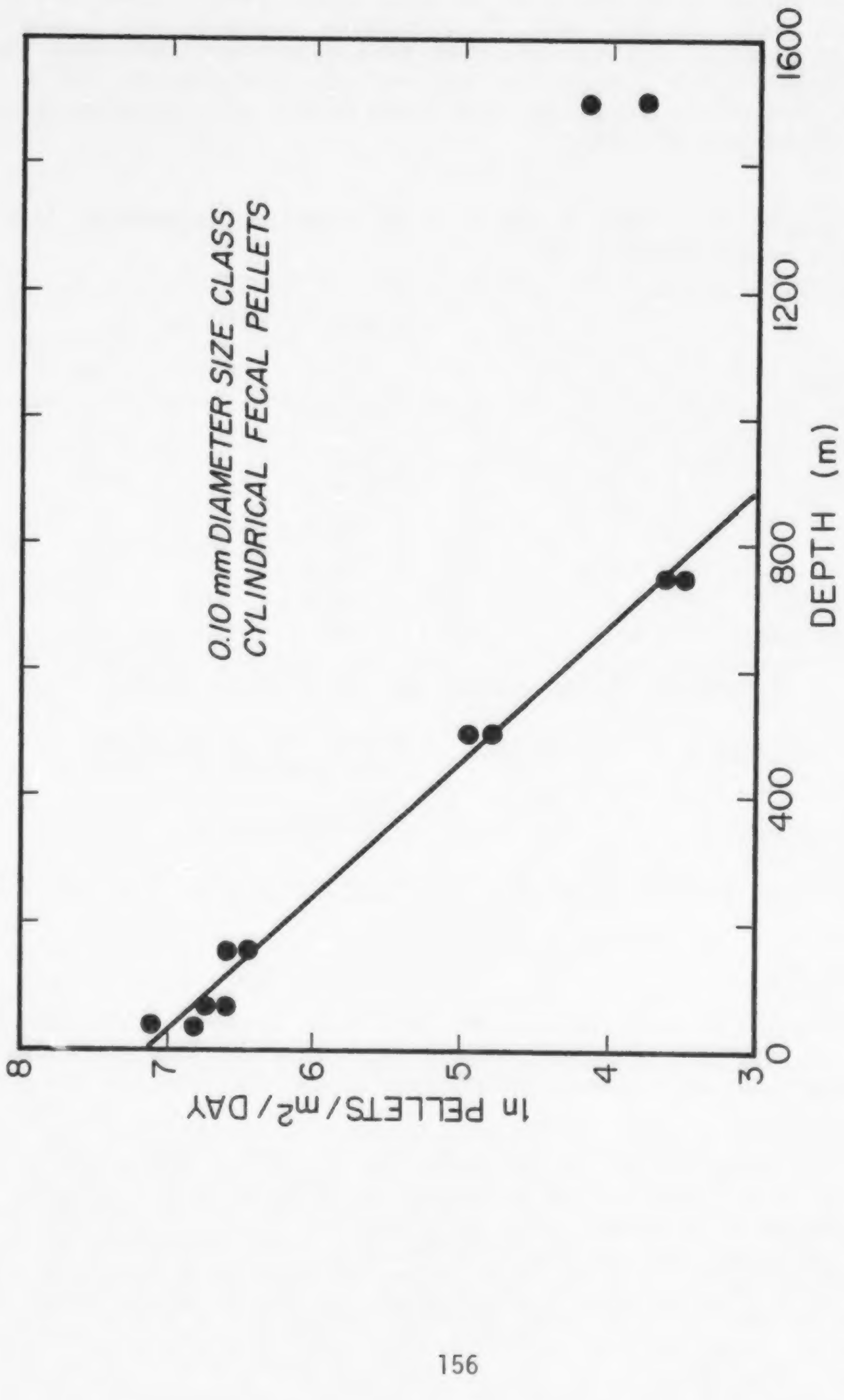

0.10 mm DIAMETER SIZE CLASS
CYLINDRICAL FECAL PELLETS
ln PELLETS/m²/DAY
8
7
6
5
4
3
0
400
800
1200
1600
DEPTH (m)

Figure 4.

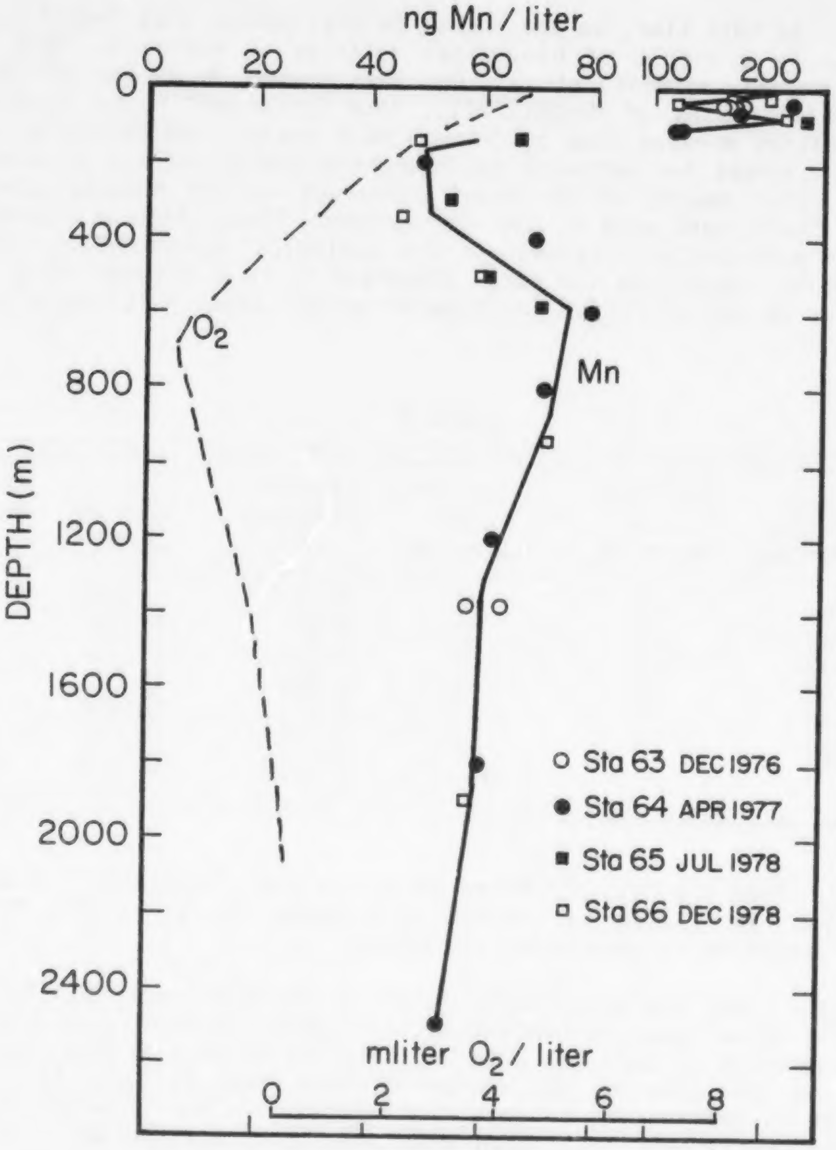

ng Mn / liter
0
20
40
60
80
100
200
0
400
800
1200
1600
2000
2400
DEPTH (m)
O2
Mn
○ Sta 63 DEC 1976
● Sta 64 APR 1977
■ Sta 65 JUL 1978
□ Sta 66 DEC 1978
mliter O2 / liter
0
2
4
6
8

Figure 5.

Figure 6. Illustrates the distribution of three Mn fractions found in the MULTIPITs with depth: the particulate, the residual particulate digested in HF (inorganic), and the soluble. In the case of Mn, most of this element resides on the particulate phase. Comparison of the dissolved Mn (Figure 5) with the total MULTIPIT Mn (this figure) suggests a loss of Mn from the latter phase, while the dissolved phase increases in the oxygen minimum zone (750 m).

Table 8. At this time, we are unable to distinguish what fraction of the total Mn flux is a result of biological activity or inorganic input (e.g., runoff, resuspended sediment, etc.). For this reason, Mn fluxes are reported for "Total" (all phases of Mn/MULTIPIT), "Mud Corrected" (i.e., hand waving using Al/Mn ratios derived from marine sediment analysis which can be used to theoretically correct for sediment inclusions--probably not too accurate) and "Soluble Mn" (the amount of Mn found dissolved in the density gradient). "Soluble" Mn fluxes were used to compute residence times since we assumed that this fraction most probably represents the biological contribution. In this table, "Total Mn" represents the total dissolved Mn in a 35-meter water column (e.g., Average Mn concentration in 35-meter water column = 157 $\mu g/m^3$ x 55 = 5,500).

TABLE 8

Depth (m)	Total Mn Flux $\mu g/m^2$/day	Mud Corr. Flux $\mu g/m^2$/day	Sol. Mn Flux $\mu g/m^2$/day	Depth Interval (m)	Total Mn $\mu g/m^2$	(Sol.) Res. Time (Years)
35	240	95	69	0-35	5,000	0.22
65	330	70	55	0-65	11,000	0.55
150	340	67	31	0-150	21,000	1.9
500	190	68	8.0	0-500	40,000	14
750	160	76	9.7	0-750	58,000	16
1,500	250	0	8.9	0-1,500	110,000	34
			(~9.0)	0-3,500	210,000	~64

Table 9. From the data presented above, we have calculated "Soluble" and "Mud Corrected" Mn fluxes to compare with Bender *et al*. (1970) Mn fluxes needed for "excess Mn in open-ocean sediments."

Table 10. This table presents total ng Pb/individual MULTIPIT (i.e., particulate + dissolved), Pb concentration in this fraction ($\mu g/g$ dry wt), Pb fluxes, and rates of change. As with Mn, total Pb appears to increase rapidly with depth to the area of the oxygen minimum zone followed by a marked decrease. It is interesting to note that except for the 35-meter collection depth, Pb concentrations associated with the total particulate flux remain essentially constant. This appears to be at odds with the literature in terms of Pb^{210} activity, since there is evidence that no regeneration of this nuclide occurs in the water column (Bacon *et al*. 1976). If this is so, then as particle mass decreases (as it does here--see Figure 3) and if Pb is retained on the particles (i.e., not regenerated), then the concentration

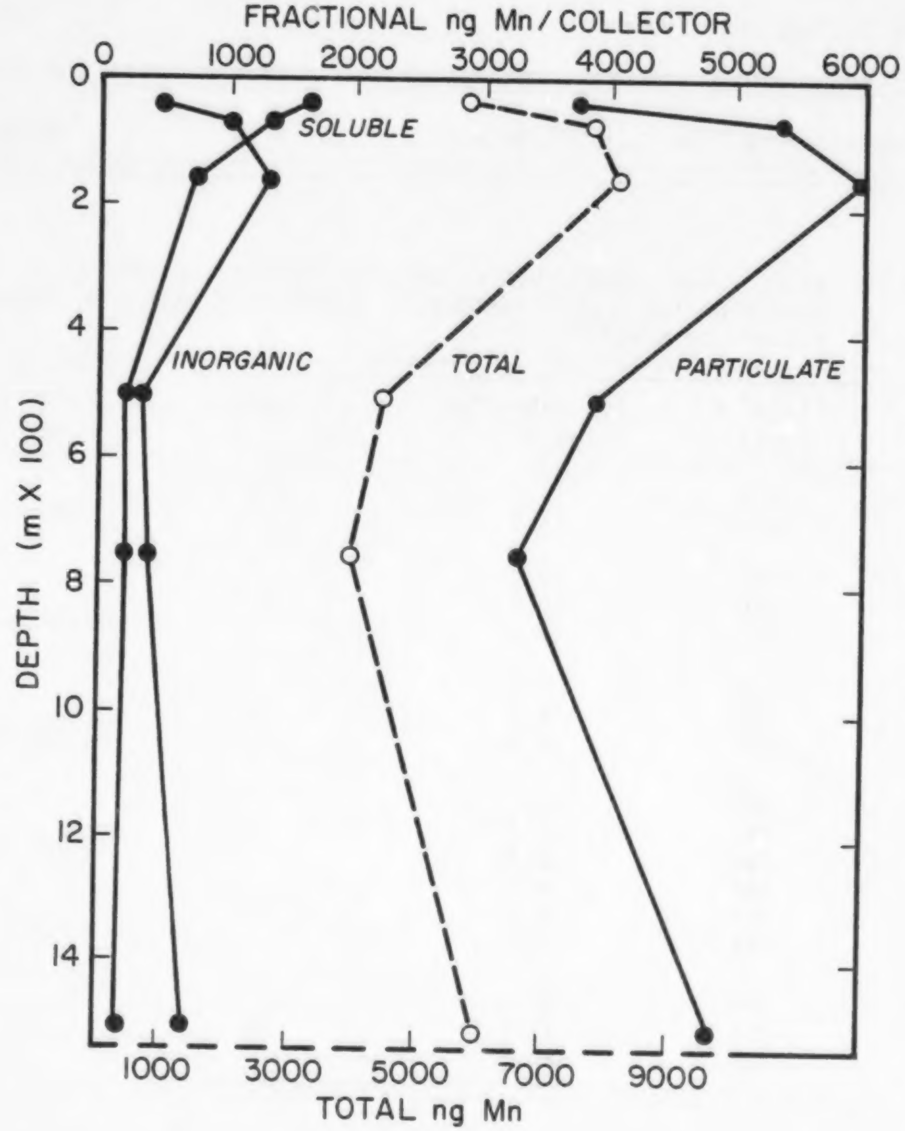

FRACTIONAL ng Mn / COLLECTOR
0
1000
2000
3000
4000
5000
6000
0
2
4
6
8
10
12
14
DEPTH (m X 100)
SOLUBLE
INORGANIC
TOTAL
PARTICULATE
1000
3000
5000
7000
9000
TOTAL ng Mn

Figure 6.

TABLE 9. MANGANESE FLUXES (from Knauer and Martin)

	μg Mn/cm^2/1,000 Years
Flux Needed for Excess Mn in Open-Ocean Sediments (Bender *et al*. 1970)	800
Soluble Mn Flux at 1,500 m	320
$\bar{x}$ Mud Corrected Flux (35, 65, 150, 750 m)	2,600
Total Uncorrected Flux at 1,500 Meters	9,100

TABLE 10. TOTAL LEAD (PARTICULATE AND DISSOLVED) COLLECTED / MULTIPIT, CONCENTRATION (SALT REMOVED). FLUXES AND RATES OF CHANGE (from Knauer and Martin)

Depth (m)	Total P (μg)	Concentration (μg g^{-1})	Fluxes μg m^{-2} day^{-1}	Ranges of Change ng l^{-1} yr^{-1}
35	529	19.0	21	
	503	17.9		(35-65) 0
65	526	39.8		
	520	26.1	21	
	500	27.0		(65-150) 16
150	643	35.9		
	606	36.9	25	
	573	36.9		(150-500) 12
500	261	32.2		
	401	31.0	14	
	339	38.9		(500-750) 5.5
750	240	48.0		
	322	35.4	10	
	168	24.7		(750-1,500) 1.4
1,500	248	20.3		
	264	39.4	13	
	420	32.3		

should increase with depth. Certainly, the common Pb isotope should not be expected to act differently from Pb^{210}. We expect to have Pb^{210} data for the MULTIPIT set in the future. It should be noted that in Knauer *et al*. (1979), Pb^{210} activity did increase with depth to 700 m during the coastal upwelling episode.

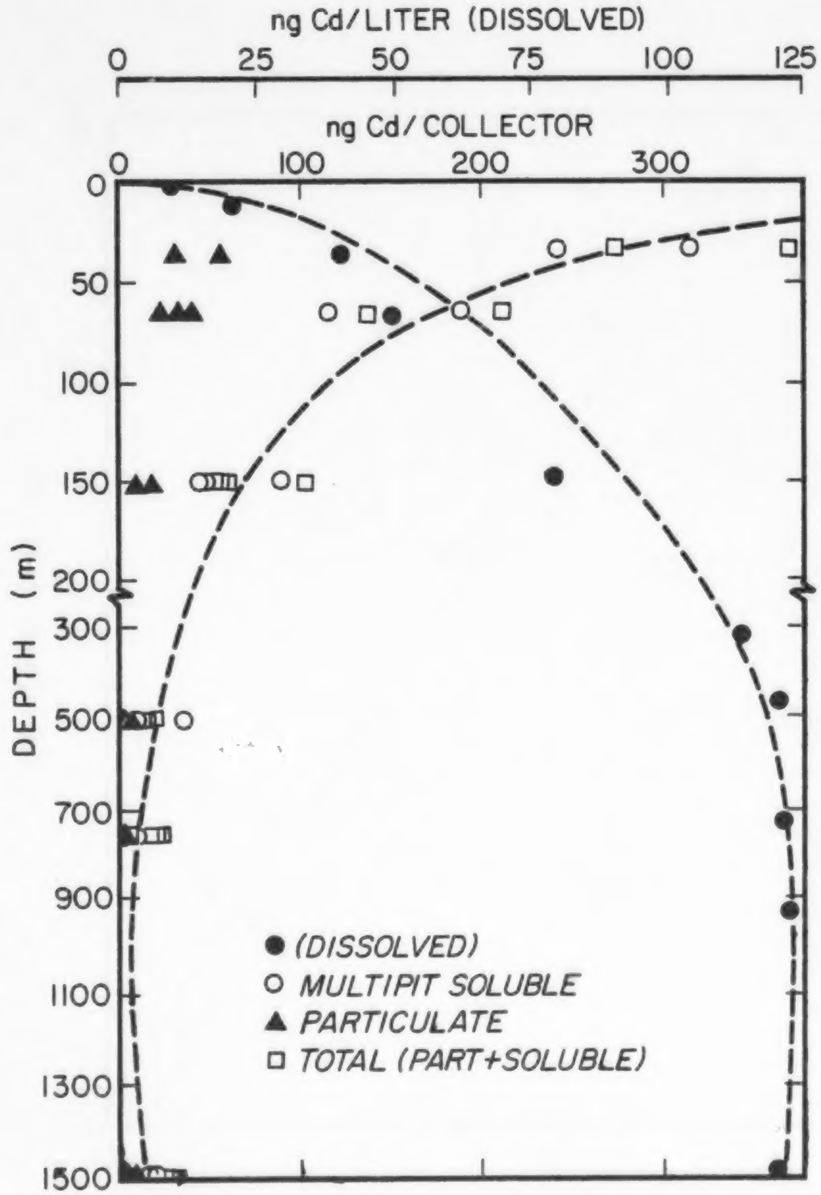

ng Cd/LITER (DISSOLVED)
0
25
50
75
100
125
ng Cd/COLLECTOR
0
100
200
300
DEPTH (m)
0
50
100
150
200
300
500
700
900
1100
1300
1500
● (DISSOLVED)
○ MULTIPIT SOLUBLE
▲ PARTICULATE
□ TOTAL (PART+SOLUBLE)

Figure 7.

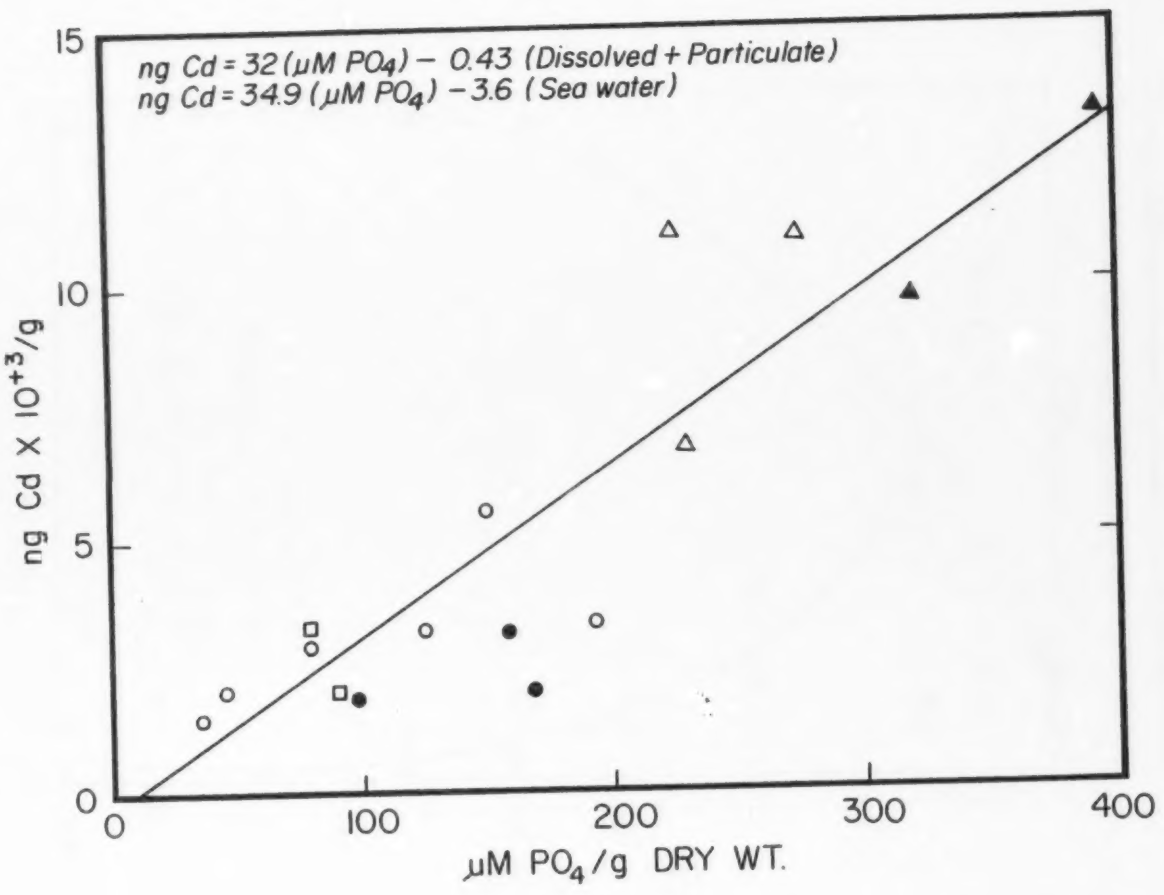

ng Cd = 32 (µM PO4) − 0.43 (Dissolved + Particulate)
ng Cd = 34.9 (µM PO4) − 3.6 (Sea water)
ng Cd X 10+3/g
µM PO4/g DRY WT.
0
5
10
15
0
100
200
300
400

Figure 8.

Figure 7. This graph compares total Cd (particulate + soluble) with "dissolved" Cd in sea water. (Again, "dissolved" Cd is defined here as that fraction left in solution after passage through a 0.4 μ acid washed nucleopore filter). The two phases appear to be related inversely, as might be expected. For example, as total Cd (from MULTIPITs) decreases with depth (squares), "dissolved" Cd (solid circles) increased with depth. This relationship is reinforced in Figure 8.

Figure 8. This is a regression of total Cd (particulate + soluble) against total PO_4^{-3} (particulate + soluble) from the MULTIPIT collections. The equation of the line [ng Cd = 32(μM $PO_4^{\overline{-}}$) - 0.43] is very similar to that published by Bruland *et al*. (1979) for the relationship between these two elements "dissolved" in sea water, which tends to support the relative accuracy of the system.

REFERENCES

Anonymous. 1977. Ocean Research in the 1980s, Center for Ocean Management Studies, University of Rhode Island. Kingston, Rhode Island, USA.

Arrhenius, G. O. S. 1963. Pelagic sediments, p. 655-727. *In* M. N. Hill (ed.), The Sea, Vol. 3. Interscience Publ., New York.

Bacon, M. P., A. W. Spencer, and P. G. Brewer. 1976. ^{210}Pb/^{226}Ra and ^{210}Po/^{210}Pb disequilibria in sea water and suspended particulate matter. Earth Planet. Sci. Lett. *32*:277-296.

Beasley, T. M., M. Heyraud, J. J. W. Higgo, R. D. Cherry, and S. W. Fowler. 1978. ^{210}Po and ^{210}Pb in zooplankton fecal pellets. Mar. Biol. *44*:325-328.

Benayoun, G., S. W. Fowler, and B. Oregioni. 1974. Flux of cadmium through euphausiids. Mar. Biol. *27*:205-212.

Bender, M. L., T-L. Ku, and W. S. Broeker. 1970. Accumulation rate of manganese in pelagic sediments and nodules. Earth Planet. Sci. Lett. *8*:143-148.

Bishop, J. K. B., J. M. Edmond, D. R. Kitten, M. P. Bacon, and W. B. Siler. 1977. The chemistry, biology, and vertical flux of particulate matter from the upper 400 M of the equatorial Atlantic Ocean. Deep-Sea Research 24:511-548.

Boothe, P. N., and G. A. Knauer. 1972. The possible importance of fecal material in the biological amplification of trace and heavy metals. Limnol. Oceanogr. *17*:270-274.

Boyle, E. and J. M. Edmond. 1975. Copper in surface waters south of New Zealand. Nature *253*:107-109.

Boyle, E. A., F. Sclater, and J. M. Edmond. 1977. The distribution of dissolved copper in the Pacific. Earth Planet. Sci. Lett. 37:38-54.

Bruland, K. W., G. A. Knauer, and J. H. Martin. 1978a. Zinc in northeast Pacific waters. Nature 271:741-743.

Bruland, K. W., G. A. Knauer, and J. H. Martin. 1978b. Cadmium in northeast Pacific waters. Limnol. Oceanogr. 23:618-625.

Bruland, K. W., R. P. Franks, G. A. Knauer, and J. H. Martin. 1979. Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel at the nanogram per liter level in sea water. Anal. Chem. Acta 105:233-245.

Cherry, R. D., S. W. Fowler, T. M. Beasley, and M. Heyraud. 1975. Polonium^{210}: Its vertical oceanic transport by zooplankton metabolic activity. Mar. Chem. 3:105-110.

Conover, R. J. 1971. Some relations between zooplankton and Bunker C oil in Chedabucto Bay following the wreck of the tanker Arrow. J. Fish. Res. Bd. Can. 28:1327-1330.

Elder, D. L. and S. W. Fowler. 1977. Polychlorinated biphenyls: Penetration into the deep ocean by zooplankton fecal pellet transport. Science 197:459-461.

Ferrante, J. G. and J. I. Parker. 1977. Transport of diatom frustules by copepod fecal pellets to the sediments of Lake Michigan. Limnol. Oceanogr. 22:92-98.

Fowler, S. W. 1977. Trace elements in zooplankton particulate products. Nature 269:51-53.

Higgo, J. J. W., R. D. Cherry, M. Heyraud, and S. W. Fowler. 1977. Rapid removal of plutonium from the ocean surface layer by zooplankton fecal pellets. Nature 226:623-624.

Honjo, S. 1976. Coccoliths: production, transportation and sedimentation. Mar. Micropaleont. 1:65-79.

Knauer, G. A., J. H. Martin, and K. W. Bruland. 1979. Fluxes of particulate carbon, nitrogen, and phosphorus in the upper water column of the Northeast Pacific. Deep Sea Res. 26(1A):97-108.

Lowman, F. G., T. P. Price, and F. A. Richards. 1971. Accumulation and redistribution of radionuclides by marine organisms, p. 161-199. In Radioactivity in the Marine Environment, National Acad. Sci.

Manheim, F. T., J. C. Hathaway, and E. Uchupi. 1972. Suspended matter in surface waters of the northern Gulf of Mexico. Limnol. Oceanogr. 17:17-27.

Martin, J. H. and G. A. Knauer. 1973. The elemental composition of plankton. Geochem. Cosmochim. Acta 37:1639-1653.

Martin, J. H., K. W. Bruland, and W. W. Broenkow. 1976. Cadmium transport in the California Current, p. 159-184. In Windom, H. L. and R. A. Duce (eds.), Marine Pollutant Transfer. D.C. Heath and Co. Lexington, Mass.

Menzel, D. W. 1974. Primary productivity, dissolved and particulate organic matter and sites of oxidation of organic matter, p. 659-678. In Goldberg, E.D. (ed.), The Sea, Vol. 5. John Wiley.

Moore, R. M. and J. D. Burton. 1976. Concentrations of dissolved copper in the eastern Atlantic Ocean 23°N to 47°N. Nature 264:242-243.

Redfield, A. C., B. H. Ketchum, and F. A. Richards. 1963. The influence of organisms on the composition of sea water, p. 26-77. In M. H. Hill (ed.), The Sea, Vol. 2. John Wiley.

Roth, P. H., M. M. Mullin, and W. H. Burger. 1975. Coccolith sedimentation by fecal pellets: laboratory experiments and field observations. Bull. Geol. Soc. Am. 86:1079-1084.

Schrader, H. J. 1971. Fecal pellets in sedimentation of pelagic diatoms. Science 174:55-57.

Small, L. F. and S. W. Fowler. 1973. Turnover and vertical transport of zinc by the euphausiid Meganyclephanes norvegica in Ligurian Sea. Mar. Biol. 18:284-290.

Small, L. F., S. W. Fowler, and S. Keckes. 1973. Flux of zinc through a macroplanktonic crustacean. Symp. on the interaction of radioactive contaminants with the constituents of the marine environment, Seattle, 1972. Int. Atomic Energy Agency.

Soutar, A., K. W. Bruland, S. A. Kling, P. A. Crill, and E. Duffrin. 1977. Monitoring the marine environment: an approach through sedimentation. Nature 266:136-139.

Spencer, D. W., P. G. Brewer, A. Fleer, S. Honko, S. Krishnaswami, and Y. Nozaki. 1978. Chemical fluxes from a sediment trap experiment in the deep Sargasso Sea. J. Mar. Res. 36:493-523.

Vinogradov, M. E. 1961. Feeding of the deep-sea zooplankton. Cons. Perm. Internat. Explor. Mer. Rapp. Proc. Reunions 153:114-120.

Votinzev, K. K. 1953. On the rate of regeneration of nutrient salts during the decomposition of Melosira barcalensis Wesl. Kodl. Akad. Nouk 92(3).

Wiebe, P. H., S. H. Boyd, and C. Winger. 1976. Particulate matter sinking to the deep-sea floor at 2,000 M in the tongue of the ocean, Bahamas, with a description of a new sedimentation trap. J. Mar. Res. 34:341-354.

CORRELATION BETWEEN DOSE LOADS IN FISH AND THE BIOGEOCHEMISTRY OF ARTIFICIAL RADIONUCLIDES IN A MARINE ENVIRONMENT

by

I. A. Shekhavona and A. P. Panarin
All-Union Scientific Research Institute
of Marine Fisheries and Oceanography
VNIRO, Moscow

The entire process of establishing life on Earth has taken place under the effects of ionizing radiation, caused by cosmic radiation and natural radionuclides from the planets. The relative constancy and stability of natural radiation has helped contemporary animals and plants adapt to this factor; however, we can assume that this type of adaptation is limited.

Scientific-technological progress, the development of the atomic energy industry, and the wide use of nuclear energy since the middle of the current century have caused the appearance of new, anthropogenic sources of ionizing radiation. In contrast with natural radiation, the concentration of artificial radionuclides is increasing in some areas of the biosphere, and the intensity of irradiation of living organisms is also changing as a consequence.

Like any other factor, after reaching a certain intensity which exceeds the limits of the tolerance developed in the course of evolution, ionizing radiation can become a limiting factor and can have a negative effect on the most radiosensitive organisms. This is also true of aquatic biocenoses, since almost all radioactive substances of an artificial origin which enter the atmosphere finally become concentrated in the world's oceans.

The ban on nuclear tests in three environments as a result of the Moscow pact of 1963 sharply decreased the intensity of global radioactive fallout, but did not totally stop artificial radionuclides from entering the hydrosphere. This was related to a number of causes. First, the existence of stratospheric reserves of long-lived products of nuclear explosions which, based on many estimates, will fall out until the year 2000 and the continuous migration of accumulated reserves of these products from land into the seas and oceans with drainage from rivers. Second, the continuation of nuclear arms tests in some countries (China, France). Third, the practice of dumping radioactive wastes in the oceans, seas, rivers, and inland bodies of water in some countries (U.S., England, Japan, Belgium, etc.) (Nelepo 1970; Patin 1970; Gromov and Spitsyn 1975).

An extensive program has recently been established for constructing nuclear power plants (NPP) on the coasts of seas and oceans, and the various aspects of building floating NPP on special platforms in coastal waters around large cities has been discussed (Gusev 1975). Projected annual production of atomic energy will increase to $2 \cdot 10^{12}$ W (el.) by the year 2000, and the number of operating NPP will increase to 5,000 versus 187 in operation in 1976 (Anonymous 1977). In these circumstances, it is logical also to expect a corresponding increase in the amount of radioactive waste. This creates the basis for local pollution of individual bodies of water by radionuclides. However, as a result of the large-scale circulation and integral biological structure of the world's oceans, regional anomalies in any one part can be reflected in the radiation situation of neighboring regions and the entire system as a whole. For this reason, any type of pollution in surface waters has become one of the most acute international problems and requires rigorous regulation.

There currently are no unified common standards for admissible concentrations of artificial radionuclides in surface waters based on sanitary-hygienic and ecological aspects in our country and abroad.

Based on current sanitary-hygienic standards for regulating the concentration of radionuclides in water, only criteria for estimating the significance of contamination of drinking water have usually been established and the basic biological chains for migration of radionuclides from the water into the human body have been taken into consideration. Such an approach evidently meets all requirements for radiation-hygienic safety, but does not include the effect of ionizing radiation and its consequences on the inhabitants of an aquatic environment, for example, fishes. The urgency of the problem of ecological, piscicultural standards for artificial radionuclides in open bodies of water has been emphasized repeatedly (Polikarpov 1964; Egami 1973; Anonymous 1976), since fish breeding more significantly suffers from deterioration of the quality of the aquatic environment. For this reason, it is of particular interest in solving the scientific and practical problems related to protecting bodies of water from pollution.

This position is probably complicated by the long-standing opinion that if hygienic-sanitary standards are satisfactory for man, then they are also suitable for fish. This is probably true in conditions of equivalent dose loads on humans and fish with some limit to the concentration of radionuclides in the water. However, in reality, the radiation loads on fish, which constantly live in water, and man, who is exposed for limited periods of time, differ. This is primarily related to the specific properties of the aquatic environment in which intensive concentrations of radionuclides up to exceedingly high levels are observed not only in fish, but also in important sources of external irradiation of fish--bottom sediment and algae--parallel to dilution and dispersion of radionuclides.

The behavior of radioactive elements in a body of water is much more complex than in the atmosphere. Their migration and concentration are a function of both the physical-chemical and biogeochemical properties of the aquatic environment which contains a large amount of dissolved and colloidal organic substances, hydrobionts and suspensions which differ in composition and origin. In addition, the rate of radionuclides in a body of water is also a function of the properties of the radionuclides themselves.

Most of the artificial radionuclides which enter an aqueous environment are isotopes of stable macro- or microelements which form a structural base for living organisms or are included in the composition of biologically active compounds which participate in all vital processes. Because of this, they are actively exchanged, assimilated, and transported by hydrobionts and their detritus, thus causing biocirculation of radioactive substances in the water.

Plankton organisms whose biomass is most significant in the world's oceans play a particularly large role in the biogenic transport of radionuclides. These inhabitants of an aquatic environment very effectively remove radioactive elements from the water due to their large adsorbing surface. During migration, plankton organisms transport the radioisotopes which they have accumulated from the surface to the lower layers of a body of water; after they die, they sink to the bottom and become concentrated in the sediment. Biogenous sediment of radionuclides in the composition of plankton residue, characterized by continuous and frequently changing generations, can result in a marked increase in their concentration in the upper layer of bottom deposits with all of the consequences for the living inhabitants of the bottom area of a body of water.

Fish also participate in the biological migration of radionuclides, but their role is insignificant. They disperse radionuclides in feeding and digestion. At the same time, in contrast with land animals, fish can accumulate and disperse dissolved radionuclides not only along the food chain, but also directly from the water. The nature and rhythm of the uptake varies significantly as a function of abiotic and biotic factors, the species, age, and the physiological condition of the fish (Shekhanova 1978). For example, it has been found that an increase in the temperature of the water involved a proportional increase in the rate and even the degree of accumulation of radionuclides by fish (Kulikov *et al*. 1978; Katkov *et al*. 1978). Such factors should apparently be considered in using heated water from NPP in breeding fish.

Particularly intensive uptake of radioactive isotopes by fish is observed when there is a shortage of some necessary components in a region and they are present in the surrounding environment. Both of these processes--feeding and digestion on the one hand, and interaction with the elements in the surrounding environment on the other--do not exclude but only supplement each other. As a result, fish accumulate artificial radionuclides in amounts which are significantly higher than their concentration in the water. Due to these, weighable dose loads are formed in their tissues and organs even when the concentrations of radionuclides are low. We emphasize that almost all artificial radionuclides are selectively accumulated and are often localized in tissues which are not used for standardization in sanitary-hygienic evaluations. However, the same tissues and organs can be critical (most radiosensitive) for the fish themselves.

In addition to plankton, the suspended matter in the world's oceans includes a significant amount of mineral particles of terrigenous and volcanic origin; the specific surface of the suspended matter is significantly greater than in living organisms. This is the reason for its excessively high adsorbing capacity. It is believed that migration of such microelements as Zn, Pb, Bi, Cu, Hg, Ag, and Mo (Kranskopf 1956) into the depths of the oceans is basically due to sorption of suspended matter by mineral components; this is apparently also true of the radioactive isotopes of these elements. Abiogenetic suspended matter together with adsorbed radionuclides is gradually transported to the lower layers of the ocean and deposited on the bottom. This process takes place with particular intensity in coastal zones where formation of sediment occurs much more rapidly than in pelagic areas. We also note that solid particles and colloids are very effectively assimilated by living organisms and are thus part of the biosedimentation system.

As a result, both sedimentation and suspended matter of mineral origin and biosedimentation affect the enrichment of the upper layer of bottom deposits with radioactive nuclides. The bottom deposits of oceans and seas in turn are good natural sorbents with inherent ion exchange, chemosorption, physical (or molecular) sorption, etc. For this reason, radionuclides are intensively and sometimes irreversibly trapped by these deposits.

Bottom sediments consisting of argillaceous minerals, sludge characterized by high dispersity and sorptive capacity, are distinguished by the strongest absorptive capacity (Gromov and Spitsyn 1975; Ryndina 1970; Garroll 1959). In addition, benthos organisms and attachment of algae which intensively assimilate them in life and convert them to soil in dying also promote the process of radionuclide accumulation in the upper layer of bottom sediments (Ketchum 1960; Schäfer 1960). The accumulation coefficients for radionuclides in such soils can reach hundreds and thousands. As a result, demersal eggs and bottom fish will be exposed to very appreciable dose loads due to gamma and even beta stratification. Aquatic plants, which accumulate radionuclides up to levels which significantly exceed their concentration in the water, cause an additional dose load for phytophilic eggs.

The radioactive substances which enter bodies of water are thus very rapidly involved in the hydrological, physical-chemical, and biogeochemical processes occurring in the water. In the final analysis, this results in the distribution and redistribution of radionuclides among the abiotic and biotic components of the aquatic environment. The role of these factors in the distribution and concentration of the individual radionuclides differs and is primarily a function of their individual properties. Sedimentation and biological factors apparently have no significant effect on the distribution of ^{90}Sr and ^{137}Cs in the ocean, since these radionuclides are primarily present in a marine environment in a dissolved state and are almost unrelated to inorganic suspensions and hydrobionts. At the same time, for ^{144}Ce, ^{95}Zr, ^{95}Nb, ^{106}Ru-^{106}Ru and particularly the isotopes of biogenous elements (^{55}Fe, ^{65}Zn, ^{54}Mn, ^{60}Co and others), the role of sedimentation and biogeochemical processes in the migration and spatial distribution can be comparable to the effects of hydrological factors (Svedov and Patin 1968).

Some authors (Timofeyeva-Resovskaya and Timofeyeva-Resovskaya 1960) believe that artificial radionuclides should be divided into four groups as a function of their behavior and distribution in a body of water: (1) hydrotropic, persisting in high concentrations in the water; (2) biotropic, intensively adsorbed by hydrobionts; (3) subtropic, accumulating primarily in bottom sediment; and (4) eurytropic, equally distributed among the individual components of the body of water.

This type of division should be useful in determining the dose loads in fish, since they are not only determined by the value of the internal irradiation from incorporated radionuclides, but also by a series of external sources of ionizing radiation which are active in the body of water--the water, water flora, the soil. In turn, the individual role of each of these sources of external irradiation in forming the integral dose load in fish will be determined by the ecology, more precisely the biotope, in which the fish live. For example, all other conditions being even, the irradiation to which pelagic and phytophilic (or bottom) eggs are exposed is far from equivalent.

As a consequence, in developing standards for monitoring radioactive contamination of bodies of water with respect to ecology and fish breeding, it is insufficient to limit the concentration of radionuclides in the water alone, but their concentration in all of the components of the body of water and particularly their concentration in aquatic plants and bottom sediments should also be taken into consideration.

We evaluated the hygienic studies of the liminal concentrations proposed by Gusev (1975) with respect to their admissibility for fish breeding to illustrate a possible ecological approach to standardization of the concentration of artificial radionuclides in sea water. In solving this problem, we analyzed the level of internal beta radiation of fish from the radionuclides accumulated in their tissues, and the extermal gamma and beta radiation from radionuclides accumulated in bottom deposits.

The doses were calculated according to the commonly used equations (Aglintsev *et al*. 1962; Khayn and Braunell 1958):

$$P_\beta = 2.13 \cdot \bar{E} \cdot C, \qquad (1)$$

where P_β is the tissue dose rate, rad/hr,

$\bar{E}$ is the mean energy of beta radiation per disintegration, Mev,

C is the concentration of radionuclides in the tissue, c/g,

$$P_\gamma = \frac{2\pi \cdot C \cdot K_\gamma}{\mu} [1 - \phi(\mu l)], \qquad (2)$$

where P_γ is the gamma radiation dose rate on the surface of the bottom, r/hr,

C is the concentration of radionuclides in the bottom sediment, c/cc,

K_γ is the gamma constant for the radionuclide, $r \cdot cm^2/g \cdot ms$,

μ is the linear attenuation coefficient for gamma quanta (broad beam) in the emitter material, cm^{-1},

l is the thickness of the irradiated layer, cm,

ϕ is King's function.

Equation (2) is recommended for an extended plane source of finite thickness. In our case, with a bottom thickness containing gamma-irradiating radionuclides equal to 20 cm, $\phi(\mu l) \rightarrow 0$ and expression (2) with an error of +1 to +10% (as a function of the energy of the gamma irradiation) approaches the expression for a semi-infinite 2π source:

$$P_\gamma = \frac{2\pi \cdot C \cdot K_\gamma}{\mu}, \text{ P/v} \qquad (3)$$

The dose of beta irradiation on the surface of the bottom was set as equal to $0.5D_\beta$ (Khayn and Braunell 1958), where D_β is the corresponding dose in the interior of the bottom sediment.

The dose rate of gamma radiation in the bottom sediment is a function of the level of accumulation and the nature of the distribution of the radioactive substances in the groups which form the bottom, and this in turn is determined by an entire series of conditions (Marey 1976; Ryndina 1970). To simplify the calculations, we assumed that the radionuclides were evenly distributed in the 20-cm surface layer of bottom sediment. This assumption could apparently be correct (at least for some long-lived radionuclides) in estuaries and coastal zones of oceans and seas (Patel et al. 1975). The fact that the concentrations of ^{60}Co and ^{137}Cs at a depth of 0.5 m did not differ from the concentrations on the surface in bottom deposits in the Columbia River, where nuclear reactor wastes have been dumped for a long time (Haushild et al. 1973), supports this assumption.

We used the upper limits of the concentrations of radionuclides in sea water (Gusev 1975) and the limiting coefficients for accumulation of radioactive and stable isotopes in fish tissues for calculating the mean doses absorbed by the tissues of fish (Bakunov et al. 1973; Gusev 1975; Patin and Petrov 1973; Pertsov 1973; Rozhanskaya 1970). Since we were interested in the dose loads in the fish itself and not in its consumer, we did not divide the accumulation coefficients into bone and muscle tissue, but took the maximum value for both types of tissue (Table 2). The expediency of this approach is supported by the fact that the actual dose of some radionuclides e.g., in the kidneys of fish, is significantly higher than the calculated dose based on our experimental measurements using thermoluminescent dosimeters (Shekhanova 1976). The effect of irradiation from radionuclides assimilated by the spine, which is directly adjacent to the kidneys, is present here.

On the other hand, it is not possible to prevent fish from entering the massive accumulation zone and the commercial marine invertebrate and algae business. As a consequence, the division of working limits of concentrations according to the different industrial zones loses its meaning in real conditions (see Table 1). Based on the above, it was found that the tissue dose

TABLE 1. WORKING LIMITS FOR CONCENTRATIONS OF RADIONUCLIDES IN SEA WATER (c/liter) WITH PROLONGED CONTAMINATION OF REGIONS INVOLVING DIFFERENT INDUSTRIAL APPLICATIONS (Gusev 1975)

		Production Zone			
Radionuclide	Fishing Zone	Mollusks	Crustaceans	Nutritive Algae	Mixed Industrial Zone
Chromium^{51}	$7 \cdot 10^{-10}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-8}$	$7 \cdot 10^{-9}$	$7 \cdot 10^{-10}$
Manganese^{54}	$1 \cdot 10^{-9}$	$6 \cdot 10^{-11}$	$8 \cdot 10^{-11}$	$5 \cdot 10^{-10}$	$1 \cdot 10^{-10}$
Iron^{55}	$4 \cdot 10^{-10}$	$4 \cdot 10^{-10}$	$2 \cdot 10^{-9}$	$4 \cdot 10^{-10}$	$4 \cdot 10^{-10}$
Iron^{59}	$3 \cdot 10^{-11}$	$3 \cdot 10^{-11}$	$1 \cdot 10^{-10}$	$3 \cdot 10^{-11}$	$3 \cdot 10^{-11}$
Cobalt^{57}	$5 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$3 \cdot 10^{-9}$	$4 \cdot 10^{-9}$	$3 \cdot 10^{-9}$
Cobalt^{58}	$1 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$7 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$7 \cdot 10^{-10}$
Cobalt^{60}	$4 \cdot 10^{-10}$	$9 \cdot 10^{-10}$	$3 \cdot 10^{-10}$	$4 \cdot 10^{-10}$	$3 \cdot 10^{-10}$
Zinc^{65}	$3 \cdot 10^{-11}$	$5 \cdot 10^{-11}$	$1 \cdot 10^{-10}$	$5 \cdot 10^{-10}$	$3 \cdot 10^{-11}$
Strontium^{89}	$1 \cdot 10^{-9}$	$6 \cdot 10^{-9}$	$6 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-9}$
Strontium^{90}	$4 \cdot 10^{-11}$	$2 \cdot 10^{-10}$	$2 \cdot 10^{-10}$	$4 \cdot 10^{-10}$	$4 \cdot 10^{-11}$
Yttrium^{90}	$2 \cdot 10^{-9}$	$5 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-10}$
Zirconium^{95}	$4 \cdot 10^{-10}$	$1 \cdot 10^{-8}$	$3 \cdot 10^{-9}$	$3 \cdot 10^{-10}$	$3 \cdot 10^{-10}$
Niobium^{95}	$1 \cdot 10^{-9}$	$4 \cdot 10^{-9}$	$5 \cdot 10^{-9}$	$3 \cdot 10^{-10}$	$3 \cdot 10^{-10}$
Molybdenum^{99}	$2 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$2 \cdot 10^{-9}$
Ruthenium^{103}	$1 \cdot 10^{-9}$	$5 \cdot 10^{-8}$	$5 \cdot 10^{-8}$	$3 \cdot 10^{-10}$	$3 \cdot 10^{-10}$
Ruthenium^{106}	$1 \cdot 10^{-10}$	$6 \cdot 10^{-9}$	$6 \cdot 10^{-9}$	$3 \cdot 10^{-11}$	$3 \cdot 10^{-11}$
Cadmium^{109}	$4 \cdot 10^{-8}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-8}$	$6 \cdot 10^{-10}$	$1 \cdot 10^{-10}$
Cadmium^{115}m	$5 \cdot 10^{-9}$	$1 \cdot 10^{-11}$	$1 \cdot 10^{-9}$	$8 \cdot 10^{-11}$	$1 \cdot 10^{-11}$
Cadmium^{115}	$7 \cdot 10^{-9}$	$2 \cdot 10^{-11}$	$2 \cdot 10^{-9}$	$1 \cdot 10^{-10}$	$2 \cdot 10^{-11}$
Antimony^{124}	$2 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$5 \cdot 10^{-10}$	$2 \cdot 10^{-11}$
Antimony^{125}	$7 \cdot 10^{-10}$	$5 \cdot 10^{-9}$	$7 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$7 \cdot 10^{-10}$
Iodine^{131}	$1 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	$2 \cdot 10^{-10}$	$1 \cdot 10^{-11}$	$1 \cdot 10^{-11}$
Cesium^{134}	$3 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$2 \cdot 10^{-9}$	$6 \cdot 10^{-10}$	$3 \cdot 10^{-10}$
Cesium^{137}	$6 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$3 \cdot 10^{-9}$	$1 \cdot 10^{-9}$	$6 \cdot 10^{-10}$
Cerium^{141}	$3 \cdot 10^{-9}$	$4 \cdot 10^{-9}$	$4 \cdot 10^{-9}$	$6 \cdot 10^{-10}$	$6 \cdot 10^{-10}$
Cerium^{144}	$4 \cdot 10^{-10}$	$6 \cdot 10^{-10}$	$6 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-10}$
Polonium^{210}	$5 \cdot 10^{-11}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-10}$	$4 \cdot 10^{-11}$	$4 \cdot 10^{-11}$
Plutonium^{239}	$2 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$5 \cdot 10^{-10}$	$1 \cdot 10^{-11}$	$1 \cdot 10^{-11}$

TABLE 2. DOSE RATE OF BETA IRRADIATION OF FISH FROM INCORPORATED RADIONUCLIDES*

Radionuclide	Upper Limits for concentrations in sea water, c/liter	Accumulation coefficients in fish tissues	Limits of concentration in fish tissue, c/kg	Tissue dose rate, rad/year
Iron^{59}	$1 \cdot 10^{-10}$	3,000	$3.00 \cdot 10^{-7}$	0.66
Cobalt^{58}	$2 \cdot 10^{-9}$	560	$1.12 \cdot 10^{-6}$	0.63
Cobalt^{60}	$9 \cdot 10^{-10}$	560	$5.04 \cdot 10^{-7}$	9.09
Strontium^{89}	$6 \cdot 10^{-9}$	200^{x}	$1.20 \cdot 10^{-6}$	12.84
Strontium^{90}	$2 \cdot 10^{-10}$	200^{x}	$4.00 \cdot 10^{-8}$	0.85
Yttrium^{90}	$5 \cdot 10^{-9}$	10	$5.00 \cdot 10^{-8}$	0.87
Zirconium^{95}	$1 \cdot 10^{-8}$	150	$1.50 \cdot 10^{-6}$	3.33
Niobium^{95}	$5 \cdot 10^{-9}$	100	$5.00 \cdot 10^{-7}$	0.40
Molybdenum^{99}	$1 \cdot 10^{-8}$	20	$2.00 \cdot 10^{-7}$	1.45
Ruthenium^{103}	$5 \cdot 10^{-8}$	100	$5.00 \cdot 10^{-6}$	6.04
Ruthenium^{106}	$6 \cdot 10^{-9}$	100	$6.00 \cdot 10^{-7}$	16.12
Cadmium^{115}m	$5 \cdot 10^{-9}$	5	$2.50 \cdot 10^{-8}$	0.30
Cadmium^{115}	$7 \cdot 10^{-9}$	5	$3.50 \cdot 10^{-8}$	0.23
Antimony^{124}	$2 \cdot 10^{-9}$	140	$2.80 \cdot 10^{-7}$	2.00
Antimony^{125}	$5 \cdot 10^{-9}$	140	$7.00 \cdot 10^{-7}$	1.14
Iodine^{131}	$2 \cdot 10^{-10}$	15	$3.00 \cdot 10^{-9}$	0.01
Cesium^{134}	$2 \cdot 10^{-9}$	230	$4.60 \cdot 10^{-7}$	1.66
Cesium^{137}	$3 \cdot 10^{-9}$	230	$6.90 \cdot 10^{-7}$	2.45
Cerium^{141}	$4 \cdot 10^{-9}$	100	$4.00 \cdot 10^{-7}$	1.08
Cerium^{144}	$6 \cdot 10^{-10}$	100	$6.00 \cdot 10^{-8}$	1.46

* Translator's note: Note to table illegible.

rate for the individual radionuclides reaches 16.12 rad/year with the recommended upper working limits for their concentration in sea water (see Table 2). The highest dose loads are formed by such radionuclides as ^{60}Co, ^{89}Sr, ^{103}, ^{106}Ru.

For comparison, we note that ^{40}K, which is usually the basic natural radioisotope which forms the beta activity in the body of any representative of flora and fauna (Pertsov 1973), creates a dose load in the tissues of fish which does not exceed $2.8 \cdot 10^{-2}$ rad/year (Polikarpov 1964).

Radionuclides contained in bottom deposits can be one of the most important sources of radiation for hydrobionts. The particularly appreciable effect of this source of ionizing radiation will be exercised not only on the comparatively radioresistant benthos organisms, but also on the much more sensitive bottom eggs, the young and adult specimens of marine fish which lead to a benthopelagic type of life. This is not always related to gamma radiation alone. The range of the effect of beta radiation from some radionuclides in water and the biosubstrate extends over distances comparable to and sometimes exceeding the dimensions of fish eggs, larvae, and a number of small hydrobionts, e.g., more than 1.5 cm for ^{106}Ru (E_{max} = 3.54 mev) (see Table 3).

TABLE 3. LENGTH OF THE MAXIMUM RANGE OF BETA-PARTICLES IN WATER AND IN THE BIOSUBSTRATE (Pertsov 1973)

Energy mev	Water (Biosubstrate) mm	Energy mev	Water (Biosubstrate) mm
0.1	0.14	0.9	4.10
0.2	0.45	1.0	4.80
0.3	0.84	1.2	5.47
0.4	1.29	1.4	6.56
0.5	1.77	1.6	7.76
0.6	2.27	2.0	9.84
0.7	2.78	3.0	15.3
0.8	3.31	5.0	25.8

In addition, as our direct measurements using thermoluminescent dosimeters showed, the dose field on the bottom of natural bodies of water contaminated with beta-radiating radionuclides exhibits no sharp difference at the boundary of the water-soil section, and changes much more smoothly than calculations indicate. We can assume that this is related both to the presence of a peculiar "diffusion layer" with a high concentration of radionuclides in the water itself, and to the presence of a highly active suspension within the water (soil particles, detritus, plankton organisms).

The latter could play a particularly significant role in the shallow regions of the open ocean and the shelf zone where intensive entrapment of soil particles from the bottom and intermixing of water masses take place during swells and where most aquatic animals live. In turn, suspended particles which are deposited on their skin could also be an additional source of external irradiation.

In an ecological evaluation of the admissible levels of radioactive contamination of an aquatic environment, it is thus also necessary to consider the presence of external sources of irradiation of fish in this environment and primarily the dose loads created by radionuclides concentrated in the bottom sediments (see Table 4).

In extreme conditions, fish will receive both a maximum dose of external gamma radiation from the bottom and a very high dose of internal beta radiation from incorporated radionuclides. The total dose rate from both sources of ionizing radiation will then be close to a value in the order of 20-30 rads/year as a function of the individual radionuclides. In these conditions, the bottom eggs, larvae, and young of fish can additionally be exposed to

TABLE 4. DOSE RATE OF GAMMA AND BETA RADIATION FROM RADIONUCLIDES CONTAINED IN BOTTOM SEDIMENTS

Radionuclide	Upper limits of Concentrations in Sea Water, c/kg	Limiting Coefficients for accumulation in Sand	Upper Limits of Contamination of Sand, c/kg	Dose Rate, rads/year		
				γ	β	γ + β
Chromium^{51}	$1\cdot10^{-8}$	1,000	$1.0\cdot10^{-5}$	1.16	---	1.16
Manganese^{54}	$1\cdot10^{-9}$	400	$4.0\cdot10^{-7}$	2.13	---	2.13
Iron^{59}	$1\cdot10^{-10}$	1,000	$1.0\cdot10^{-7}$	0.71	0.11	0.82
Cobalt^{60}	$9\cdot10^{-10}$	1,000	$9.0\cdot10^{-7}$	19.22	0.81	20.03
Zinc^{65}	$5\cdot10^{-10}$	60	$3.0\cdot10^{-8}$	0.11	---	0.11
Zirconium^{95}	$1\cdot10^{-8}$	150	$1.5\cdot10^{-6}$	6.40	1.67	8.07
Niobium^{95}	$5\cdot10^{-9}$	150	$7.5\cdot10^{-7}$	3.20	0.30	3.50
Ruthenium^{103}	$5\cdot10^{-8}$	600	$3.0\cdot10^{-5}$	21.35	18.18	39.53
Ruthenium^{106}	$6\cdot10^{-9}$	600	$3.6\cdot10^{-6}$	4.66	48.38	53.04
Cadmium^{109}	$4\cdot10^{-8}$	100	$4.0\cdot10^{-6}$	0.09	---	0.09
Antimony^{124}	$2\cdot10^{-9}$	300	$6.0\cdot10^{-7}$	4.85	2.15	7.00
Cesium^{134}	$2\cdot10^{-9}$	300	$6.0\cdot10^{-7}$	4.27	1.08	5.35
Cesium^{137}	$3\cdot10^{-9}$	300	$9.0\cdot10^{-7}$	2.75	1.58	4.33
Cerium^{141}	$4\cdot10^{-9}$	1,400	$5.6\cdot10^{-6}$	0.59	7.65	8.24
Cerium^{144}	$6\cdot10^{-10}$	1,400	$8.4\cdot10^{-7}$	0.19	10.28	10.47

external beta radiation from bottom sediments which attains 48.38 rads/year for ^{106}Ru, for example, 53.04 rads/year ($\approx$ 145 mrads/day) together with the gamma components.

It is known that other aquatic fauna and flora (Chipman 1972; Anonymous 1976) as organisms representing the highest degree of development with respect to fish are the most radioresistant link in the set of hydrobionts. If we take 10-15 mrads/day (approximately 5 rads/year) (Shekhanova 1975) as the maximum admissible dose rate for irradiation of the gonads and kidneys of fish, the upper limits of the concentrations of radionuclides in sea water (Gusev 1975) will be too high from a radioecologic viewpoint not only due to the total accumulation of external (gamma) and incorporated (beta) sources of radiation, but also even in exposure to one of these sources, e.g., ^{60}Co, ^{89}Sr, ^{95}Zr, ^{103,106}Ru, ^{124}Sb, ^{134}Cs. If we consider the total contribution of these sources plus the external beta radiation from the bottom, then the following must be added to this series of emitters: ^{137}Cs, ^{141}Ce, and ^{144}Ce.

The admissible levels of irradiation for fish for the individual radionuclides can be obtained with the concentrations in sea water shown in Table 5. These concentrations were calculated with the condition that each radionuclide should not create a total dose rate of external and internal radiation exceeding 5 rads/year and this apparently corresponds to the radioecological requirements more precisely.

TABLE 5. WORKING LIMITS FOR CONCENTRATIONS OF ARTIFICIAL RADIONUCLIDES IN SEA WATER (c/liter)

	Limits of Concentrations in Sea Water	
Radionuclide	Hygienic (Gusev 1975)	Ecologic (our data)
Chromium^{51}	$1 \cdot 10^{-8}$	$1.0 \cdot 10^{-8}$
Manganese^{54}	$1 \cdot 10^{-9}$	$1.0 \cdot 10^{-9}$
Iron^{59}	$1 \cdot 10^{-10}$	$1.0 \cdot 10^{-10}$
Cobalt^{60}	$9 \cdot 10^{-10}$	$1.0 \cdot 10^{-10*}$
Zinc^{65}	$5 \cdot 10^{-10}$	$5.0 \cdot 10^{-10}$
Strontium^{89}	$6 \cdot 10^{-9}$	$2.0 \cdot 10^{-9*}$
Strontium^{90}	$2 \cdot 10^{-10}$	$2.0 \cdot 10^{-10}$
Zirconium^{95}	$1 \cdot 10^{-8}$	$3.5 \cdot 10^{-9*}$
Niobium^{95}	$5 \cdot 10^{-9}$	$5.0 \cdot 10^{-9}$
Ruthenium^{103}	$5 \cdot 10^{-8}$	$4.3 \cdot 10^{-9*}$
Ruthenium^{106}	$6 \cdot 10^{-9}$	$3.4 \cdot 10^{-10*}$
Cadmium^{109}	$4 \cdot 10^{-8}$	$4.0 \cdot 10^{-8}$
Antimony^{124}	$2 \cdot 10^{-9}$	$8.8 \cdot 10^{-10*}$
Cesium^{134}	$2 \cdot 10^{-9}$	$1.0 \cdot 10^{-9*}$
Cesium^{137}	$3 \cdot 10^{-9}$	$5.1 \cdot 10^{-10*}$
Cerium^{141}	$4 \cdot 10^{-9}$	$1.7 \cdot 10^{-9*}$
Cerium^{144}	$6 \cdot 10^{-10}$	$2.0 \cdot 10^{-10*}$

* Radionuclides limited by an ecologic criterion.

The calculation of the possible dose loads for fish is naturally approximate. The addition of gamma radiation from the water and incorporated radionuclides and the gamma and beta radiation from algae were not taken into consideration. External radiation from the radionuclides contained in the gastrointestinal tracts of fish can have a significant effect on the gonads and kidneys of the fish (Orlov *et al*. 1978). In addition, as we observed above, the doses of external radiation from the bottom shown in Tables 4 and 5 were determined according to limiting coefficients for accumulation of radionuclides in the sand on Baltic Sea beaches. However, argillaceous soil in the sea (Marey 1976; Ryndina 1970), whose sorption capacity is approximately 15 times higher than that of sand (Zlobin 1965) with all other conditions being equal, has the highest capacity to accumulate radionuclides. For example, Black Sea sludge accumulated 3.3 times more ^{106}Ru, 7.8 times more ^{137}Cs, and 10 times more ^{144}Ce than sand (Ryndina 1970). Higher doses of gamma and beta radiation from the bottom should naturally be anticipated in this case.

In actual conditions, the presence of any one radionuclide in wastewater from nuclear power plants, as well as the presence of the entire set of radionuclides examined, are not very probable. In this case, the working limits of the concentrations should be standardized not only for each individual radioisotope, but also for actual mixtures of isotopes present in the wastes from a given plant.

On the whole, the analytical evaluation of the possible doses of radiation of fish in the presence of artificial radionuclides in sea water in concentrations corresponding to those shown in Table 1 indicates the need for making these limits more precise with respect to the interests of the fish industry.

SUMMARY

The intensity of irradiation is dependent upon the concentration of artificial radionuclides in organs and tissues of fish (inner sources) and their distribution in water, vegetation, and bottom sediments (outer sources). The value of the dose of outer irradiation is associated with biogeochemistry of radionuclides in the aquatic environment. In those cases when radionuclides are accumulated in much greater quantities in bottom sediments than in water, the outer irradiation is responsible for the integral dose. The admissible concentration of some radionuclides in water bodies is recommended to be determined in relation to biogeochemical regularities of the behavior pattern of radionuclides and intensity of fish irradiation.

REFERENCES

Aglintsev, K. K., V. M. Kodvukov, A. F. Lyzlov, and Yu. V. Sivintsev. 1962. Applied dosimetry. Moscow, Gosatomizdat Press, 248 pp.

Anonymous. 1976. Effects of ionizing radiation on aquatic organisms and ecosystems. Tech. Rep. Ser. No. 172, IAEA, Vienna, 131 pp.

Anonymous. 1977. Production of atomic energy. Report of the OON Scientific Committee on the Effect of Atomic Radiation. A/AS, 83/343. 107 pp.

Bakunov, N. A., A. P. Panarin, and L. V. Fedotova. 1973. Accumulation of Cs^{137} in commercial species of fish in the Caspian Sea. In Radioecology of Aquatic Organisms, 2. Riga, Zinatne Press, pp. 229-234.

Chipman, W. A. 1972. Ionizing radiation. In Marine Ecology, a Comprehensive Integrated Treatise on Life in Oceans and Coastal Waters, 1:3. Wiley-Interscience, London-New York-Sydney-Toronto, 1578 pp.

Egami, Nabuo. 1973. Radioactivity and Fish (Japanese), Tokyo, 398 pp.

Garroll, D. 1959. Ion exchange in clays and other minerals. Bull. Geol. Soc. Amer., 70:749.

Gromov, V. V., and V. I. Spitsyn. 1975. Artificial radionuclides in the marine evironment. Moscow, Atomizdat Press, 224 pp.

Gusev, D. I. 1975. Hygienic criteria for evaluating contamination of coastal marine waters by radionuclides. Reprinted from Impacts of Nuclear Releases Into the Aquatic Environment, IAEA, Vienna, pp. 363-373.

Haushild, W. L., H. H. Stevens, et al. 1973. Radionuclides in transport in the Columbia River from Pasco to Vancouver, Washington. Geol. Surv. Proceedings IV:43.

Katkov, A. Ye., D. I. Gusev, A. V. Dzekunov, M. I. Grachev, Ye. N. Lyapin, and V. D. Stepanova. 1978. Effect of water temperature on accumulation of radionuclides in fish. In Problems in the Radioecology of Cooling Nuclear Power Plants. Svertlovsk, (UNTs AN SSSR), pp. 70-75.

Ketchum, B. H. 1960. Oceanographic research required in support of radioactive waste disposal. Disposal of radioactive waste. Vienna, 2:285-291.

Khayn, J. and T. Braunell (eds.). 1958. Radiation Dosimetry. Moscow, IL, 758 pp.

Kranskopf, K. B. 1956. Factors controlling the concentration of thirteen rare metals in sea water. Geochim. et cosmochim. acta, 9(1/2):1

Kulikov, N. V., L. N. Ozhegov, M. P. Chebotina, and V. F. Bochenin. 1978. Accumulation of radionuclides by freshwater hydrobionts at different water temperatures. In Problems in the Radioecology of Cooling Nuclear Power Plants. Svertlovsk (UNTs AN SSSR), pp. 65-69.

Marey, A. N. 1976. Sanitary conservation of water from contamination by radioactive substances. Moscow, Atomizdat Press, 222 pp.

Nelepo, B. A. 1970. Hyclear hydrophysics. Moscow, Atomizdat Press, 224 pp.

Orlov, E. V., A. P. Panarin, and I. A. Shekhanova. 1978. Formation of the dose load and effects of chronic irradiation of tilapia in strontium^{90} solutions. Moscow, Works of the VNIRO, Ecologic Aspects of Chemical and Radioactive Pollution of the Aquatic Environment, 134(2):94-104.

Patel, B., C. D. Mulay, and A. K. Ganguly. 1975. Radioecology of Bombay harbour--A tidal estuary. Estuarine and Coast. Mar. Sci., 3(1):13-42.

Patin, S. A. 1970. Radioactive contamination of the marine environment. Moscow, TsNITEIRKh, pp. 1-42.

Patin, S. A., and A. A. Petrov. 1973. Artificial radioactivity in sea water and commercial hydrobionts in the world's oceans. In Radioecology of Aquatic Organisms, 2. Riga, Zinatne Press, pp. 200-209.

Pertsov, L. A. 1973. Ionizing radiation of the biosphere. Moscow, Atomizdat Press, 288 pp.

Polikarpov, G. G. 1964. Radioecology of marine organisms. Moscow, Atomizdat Press, 296 pp.

Rozhanskaya, L. I. 1970. Manganese, copper, and zinc in water and organisms in the Sea of Azov. In Marine Radioecology. Kive, Naukova dumka Press, pp. 182-208.

Ryndina, D. D. 1970. Sorption and desorption of radionuclides by marine sediments, algae, and detritus. Ibid., pp. 46-62.

Schäfer, M. B. 1960. New research required in support of radioactive waste disposal. Disposal of radioactive wastes. Vienna, 2:267-282.

Shekhanova, I. A. 1975. Biologic and piscicultural aspects of standardization of the concentration of radioactive substances in the aquatic environment. TsNIITEIRKh, 32 pp.

Shekhanova, I. A. 1976. Biologic evaluation of the effects of radioactive contamination of the aquatic environment of fish. Moscow, VNIRO, 57 pp.

Shekhanova, I. A. 1978. The biologic role of artificial radionuclides in the ontogenesis of fish. Auth. Abst. Doct. Diss., Moscow, 53 pp.

Svedov, V. P., and A. A. Patin. 1968. Radioactivity in oceans and seas. Atomizdat Press, 288 pp.

Timofeyeva-Resovskaya, Ye. A., and N. V. Timofeyeva-Resovskaya. 1960. Tr. Ural'sk. fil. AN SSSR, No. 12, Sverdolvsk, p. 194.

Zlobin, V. S. 1965. Some features of the mechanism of contamination of bottom sediments and soils by radioactive substances. In Problems in the Radiation-Hygienic Investigation of the Sea. Moscow, Atomizdat Press, pp. 93-108.

THE CONSEQUENCES OF CHEMICAL POLLUTION OF THE "WATER-BOTTOM SEDIMENT" CONTACT ZONE IN THE SEA

A. Bronfman and Z. B. Aleksandrova
Institute of Economics, Ukrainian SSR Academy of Sciences, and
Azov Scientific Research Institute of Fisheries

It has become obvious that the direct toxic effect of pollutants is not the only channel through which they impact marine biocenoses. Indirect biologic effects caused by transformations of the chemical system in polluted waters also play an important role in this process.

Naturally, the relative importance of direct and indirect (intermediate) effects will differ in each case. In the overall context of this question, these aspects are of undisputed interest. However, the methodologically correct organization of studies of the problem should be based on biogeocenotic principles, which suggest integral consideration not only of the reactions in systems of the "pollutant-organism, population, biocenosis" type, but also changes in the chemical system of the sea which could have a direct effect on the degree of toxicity of the various pollutants or the level of resistance of marine organisms to the toxins.

Objectively, there are various circumstances which stimulate orientation of studies in this direction. They primarily include the fact that the corresponding changes in the hydrochemical system of a body of water either significantly attenuate or, on the other hand, potentiate the result of the toxic effect of pollution. This important concept is not new, but it has still been inadequately considered in experimental studies on stimulation and prediction of the biologic effects of marine pollution.

The transformation of chemical processes in polluted marine waters has been studied comparatively little, especially with respect to the contact zones with bottom sediment and the atmosphere. Vernadskiy (1960) called these specific marine biotopes "zones of condensation of life" and attributed an enormous role to them in the biologic and geochemical structures of bodies of water.

In the present report, the authors examine some aspects of this question for the "water-bottom sediment" contact zone. The oxygen regime of bottom waters and processes of the interchange of biogenic elements between bottom residue in the marine waters covering them were selected as the parameters to be examined. The result of many years of complex studies of the Azov Sea and special laboratory experiments served as the empirical basis.

Deterioration of the oxygen regime due to inhibition of photosynthesis and to significant consumption of oxygen for oxidation of organic components of wastewaters is one of the ways in which toxic substances affect biota. According to the calculations of Oertzen (1972), 3.6 million tons of oxygen are consumed annually in the Baltic Sea in the oxidation of organic matter from domestic wastewaters.

More pronounced deterioration in the oxygen regime should be expected in the bottom layers of the sea where pollutants accumulate and where aeration of the water is poor. This situation is particularly dangerous because the bottom layers of the sea are the habitat for zoobenthos and populations of many commercial fish, and because important physical-chemical processes affecting the chemical parameters and productivity of the pelagic zone occur here.

There are currently only isolated data on the consequences of pollution of the contact zone for the oxygen regime of bottom waters (Bronfman and Gorstko 1978). The urgent need for reliable information on this subject has become increasingly obvious.

Quantitative information on the effect of different pollutants on the concentration of dissolved oxygen could undoubtedly be obtained by using laboratory models of the process, but extrapolation of the experimental results to actual conditions in a real body of water involves the risk of significant error. More objective evaluations can be made on the basis of the analysis of direct natural measurements which permit consideration of a number of important physical-chemical factors whose simulation is difficult in experiments or is not possible in general.

The problem was solved for petroleum products and detergents in our study. The results of 114 synchronous measurements of different hydrochemical parameters in the Azov Sea, conducted within a broad range of fluctuations, were the basis of the calculations. Multiple regression analysis was used to determine the simultaneous effects of many parameters as well as the relative effect of each parameter.

The following empirical models were investigated:

$$K_{O_2} = f/E, (BOC_I)_{gr}, H_B, H_{gr}/$$

$$K_{O_2} = f/E, (BOC_I)_{gr}, D_B, D_{gr}/$$

$$K_{O_2} = f/E, (BOC_I)_{gr}, H_B, H_{gr}, D_B, D_{gr}/$$

where K_{O_2} is the concentration of dissolved oxygen in the layer 0.5 m from the bottom (range of values studied from 0.21 to 9.80 mg/liter);

E is the total vertical stability of the water in the surface-bottom layer (range from 19.72 to 3.85 thousand arbitrary units);

$(BOC_I)_{gr}$ is the daily biochemical oxygen consumption by the surface layer of the bottom sediment (range from 10.09 to 0.50 g O_2/m^2 per day);

H_B, H_{gr} is the concentration of petroleum products in the bottom layers (range from 0.30 to 6.0 mg/liter) and the surface layer of the sediment (range from 0.03 to 9.35 mg/g);

D_B, D_{gr} is the same for detergents (range from 0.02 to 0.75 mg/liter and from 0.03 to 6.27 mg/g).

The models satisfactorily approximate the phenomenon investigated--the multiple correlation coefficients consisted, respectively, of 0.627, 0.643, and 0.874, while the numerical values of Fisher's dispersed ratio (F) were 8.77, 11.11, and 6.67; with these degrees of freedom, the critical distribution values for F exceeded 99%.

Based on the values of Fisher's criterion and the level of significance α (Table 1), the arguments for the models based on their effect on the oxygen regime of bottom waters were distributed in the following sequence: vertical stability of the water, biochemical oxygen consumption by the contact zone of the sediment, detergents in the bottom layers of the sea, detergents and petroleum products accumulated in the surface layer of the sediment. The petroleum products contained in the bottom layers have an insignificant effect on the concentration of oxygen dissolved there, and are not a significant factor according to the criteria selected.

TABLE 1. VALUES OF FISHER'S CRITERION (F), LEVELS OF SIGNIFICANCE (α), AND REGRESSION COEFFICIENTS (a) FOR THE ARGUMENTS OF THE MODELS STUDIED

Arguments of Model	F	α	a
E	6.63 - 9.85	≧ 99%	-7.0×10^{-5} -8.4×10^{-5}
$(BOC_I)_{gr}$	6.57 - 6.82	≧ 98%	-0.20 -0.23
D_B	6.49 - 9.49	≧ 98%	-5.00 -5.66
D_{gr}	3.68 - 5.34	≧ 90%	-0.30 -0.40
H_{gr}	1.04 - 4.42	> 75%	-0.10 -0.15
H_B	0.02 - 0.03	--	-0.06

Judging by the value of the regression coefficient (a), each mg of petroleum products and detergents entering the bottom layers of the sea with other conditions being equal reduces the concentration of oxygen in these layers by 0.06 and 5.0-5.7 ml/liter, respectively. Petroleum products and detergents accumulated in the surface layer of the bottom sediment were represented, respectively, by 0.10-0.15 and 0.30-0.04 ml/liter.

Thus, even "moderate" pollution of bottom water and sediment by organic components from waste waters results in a significant decrease in the concentration of oxygen in the benthoic zone leading to asphyxiation and death of benthoic organisms. This is most evident in the case of detergents. The effect of petroleum products on the oxygen regime in the "water-bottom sediment" contact zone is relatively weak, possibly due to the entry of heavy, slowly oxidized petroleum fractions in the bottom layers of the sea.

The development of an oxygen deficiency and organic pollution of the benthoic zone is a relatively rapid process; even in the absence of any anthropogenic impurities, oxygen consumption due to biochemical oxidation of organic matter in the surface layer of bottom sediments usually takes place intensively. In particular, in clean or slightly polluted zones of the Azov Sea in the warm season of the year, oxygen consumption for oxidation of 1 g of organic matter in the sediment consists of 25-60 mg of oxygen per day (Aleksandrova and Romova 1977). The value of $(BOC_I)_{gr}$ is equal to an average of 3.7 here, attaining 10.5 g O_2/m^2 per day.

All of the above increase the need for a quantitative evaluation of the potential risk of an oxygen deficiency due to pollution of the benthoic zone of the sea. The time (T) during which the oxygen reserve in the bottom layers is completely exhausted for biochemical oxidation of organic matter accumulated in the "water-bottom sediment" contact zone on cessation of vertical water exchange can be used as the criterion. The oxygen reserve (R_{O_2}) is calculated as:

$$R_{O_2} = (K_{O_2})_{100\%} - (K_{O_2})_{60\%},$$

where $(K_{O_2})_{100\%}$ and $(K_{O_2})_{60\%}$ are the concentrations of oxygen with 100% and 60%, respectively, saturation of the water in the bottom layer.[1]

In view of the above:

$$(K_{O_2})_{100\%} - (BOC_I)_{gr} \frac{T}{r} = (K_{O_2})_{60\%},$$

---

[1] The value of 60% was selected as the upper limit for asphyxiation of organisms inhabiting the "water-bottom" contact zone.

$$\text{hence, } T = \frac{0.4\,(K_{O_2})_{100\%}\,h}{0.7(BOC_I)_{gr}},$$

where h is the height of the bottom layer used in the calculations, and 0.7 is the transition factor from weight to volume units of oxygen.

Experimental determination of time T for a given body of water can be made for "critical" phases of an oceanographic regime characterized by the maximum frequency and duration of stagnation of most of the indigenous organic matter entering the benthoic zone and the highest activity for its biochemical oxidation.

This approach was first implemented in developing a method for preserving the natural complex of the Azov Sea. The calculations (Aleksandrova and Romova 1977) were made for 20 basic oceanographic stations based on averaged data from special complex observations made in the summers of 1969-1977. The value of the biochemical oxygen consumption in the bottom layers of the sea, consisting of approximately 10% of the $(BOC_I)_{gr}$, was not considered in the calculations.

The results obtained allowed zoning the Azov Sea according to the time of potential possible depletion of the oxygen reserve in the bottom layer (Figure 1), and also establishing that the majority of the sea is characterized by a value of $T \leq 0.75$ days; it thus exhibits minimum natural resistance to organic pollution.

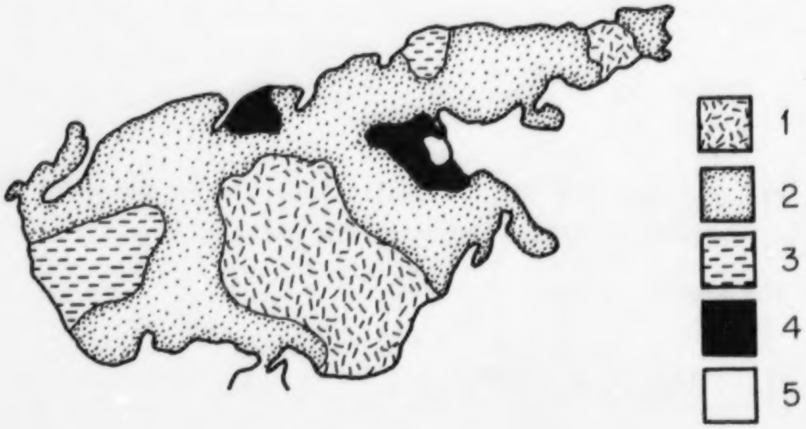


Figure 1. Zoning of the Azov Sea according to the time for potential possible depletion of the oxygen reserve in the bottom layer. 1 = $T < 0.5$ cyt.; 2 = $0.5 < T < 0.75$ cyt.; 3 = $0.75 < T < 1.0$ cyt.; 4 = $1.0 < T < 1.5$ cyt.; 5 = $T < 1.5$ cyt.

Based on an analysis of all of the data systems obtained in conducting the studies, a number of recommendations were developed to improve the system for protecting the Azov Sea from pollution. Certain additions were also introduced into the existing programs for ecological monitoring.

In the overall context of the question examined, the fact that disturbing the oxygen regime in the bottom layer of the sea, organic pollution of the bottom can result in a decrease in the exchange processes in the "water-bottom sediment" system and significant corrections can be made in the chemical balance of the bottom, is of significant interest.

Unfortunately, these studies are still in the initial stage. At the same time, the available data indicate the need to activate them, also within the framework of solving problems related to the consequences of pollution of the sea.

These findings, obtained during limnological (Hatchinson 1969; Martynova 1973; Lars 1974; Fillos and Biswas 1976; Lech 1977) and marine studies (Pirogova 1953; Yrukovskiy 1972; Fonselius 1972; Aleksandrova and Bronfman 1975; Rowe *et al*. 1975), basically can be interpreted in the form of the two major positions stated below.

1. The direction and degree of migration of biogenic elements in the "water-bottom sediment" system are decisively regulated by the concentration of oxygen, the pH value, and the oxidative-reductive conditions in the contact zone, i.e., those physical-chemical parameters which are most strongly altered under the influence of pollution.

2. Exchange of biogenic elements in the direction of the bottom $\rightleftarrows$ pelagic zone takes place relatively intensively, and in this respect should be considered an important element in the biogenic balance of the photic zone of the sea. Thus, according to the evaluations of Rowe *et al*. (1975), desorption of ammonium nitrogen from bottom sediments is equal to 14 mg/m^2 per day on the continental shelf of the ocean under conditions close to anaerobic. According to Yurkovskiy's observations (1972), desorption of phosphate phosphorus in stagnating basins in the Baltic Sea consists of 9 mg/m^2 per day.

The geochemical mechanism of exchange and its role in the biogenic balance has been more completely studied in the Azov Sea, which is the most appropriate natural model for investigating this process based on a number of morphological-structural features and the features of the physical-chemical regime. The almost annual summer stagnation of the water, characterized by extremely high indexes for the vertical stability of the layers (up to 96,000 arbitrary units), and the development of an oxygen deficiency and reducing conditions in the contact zone of the sea, is one of the important features. In individual years, iso-oxygen of 60% satuation and zero isovolts are mapped here for areas of up to 20,000 km^2, and the oxidative-reductive potential of the bottom sediments is altered in the range of 300 to -200 mV (Aleksandrova and Bronfman 1975; Bronfman 1976).

Observations showed that sorption processes, which result in impoverishment of the bottom layers and biogenic elements, primarily develop in conditions of sufficient aeration of the contact zone of the sea. In conditions of stagnation, the active desorption in the water through the reducing surface of the bottom sediment is observed. Typical situations for phosphates are illustrated in Figure 2.

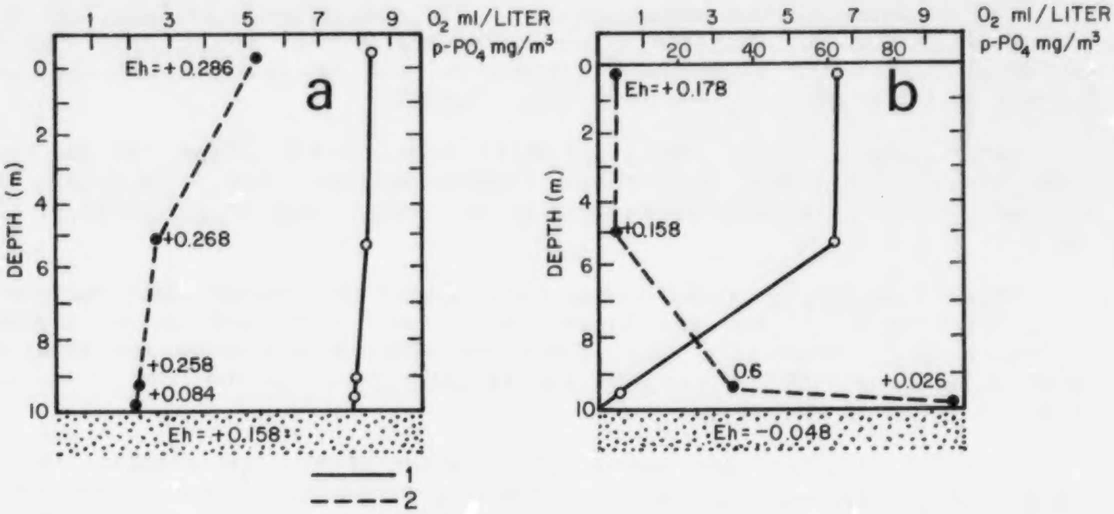


Figure 2. Vertical distribution of dissolved oxygen (1), phosphate (2), and oxidative-reductive potential with different degrees of aeration of the water in the Azov sea: (a) conditions of adequate aeration; (b) conditions of stagnation.

The results of natural observation, special laboratory studies conducted in a broad range of oxidative-reducing conditions, and also the data of Hatchinson (1969), Ayvazova and Fedosov (1972), Yurkovskiy (1973), Shippel and Hallberg (1973) and Lech (1977) permit drawing the following conclusions with respect to the basic mechanisms of exchange of nitrogen, phosphorus, and silicon compounds in the "water-bottom sediment" system.

Desorption of phosphorus compounds from the bottom sediment primarily occurs in the form of phosphates whose transition to the aqueous phase and reducing conditions of the medium is basically due either to concentration diffusion or an increase in the solubility of the phosphoric acid potassium and iron salts accumulated in the sediment. Biochemical dephosphorylation of the organic matter in the sediment also plays a determining role in this process. The identity of the physical-chemical properties of the phosphate and silicate anions forms the basis for hypothesizing that the changes in solubility and the ion exchange reactions in the SiO_3 "-Ca-Fe" system are also the basis for desorption of silicon. The yield of nitrogen is basically determined by anaerobic ammonification of organic matter with subsequent dissolution of ammonium salts.

The data on special vertical zoning of the "water-bottom" system obtained over many years allowed conducting a statistical study of the functions $\Delta P = f(Eh)$ and $\Delta P = f(K_{O_2})$, where ΔP is the difference in the concentration of phosphate in the 5 cm contact layer of water and at a distance of 0.5 m from the bottom. This index has been used as an indirect criterion of the nature and intensity of exchange.

As indicated by the results obtained, in the 20-25°C temperature range, the limit values of the oxidative-reducing potential of the surface layer of sediment and the concentration of oxygen in the contact zone of the sea which cause a change in the direction of migration of biogenic elements are, respectively, equal to 20 mV and 4.5 ml/liter (60% saturation).

The results of laboratory simulation of exchange processes have confirmed these limit values for Eh and K_{O_2} (20 mV and 3.5 ml/liter). This also allowed estimation of the diffusion coefficients for biogenic elements from the bottom sediment of the bottom layers of the sea. Based on average data, the latter consist of $4 \cdot 10^{-3}$ m^2/day for phosphates and silicates, and 10^{-2} m^2/day for ammonium.

According to the calculations performed, the mean rates of desorption of phosphate, silicon, and ammonium nitrogen from bottom residue in the Azov Sea were equal to 7, 190, and 150 mg/m^2 per day, respectively.

Together with the materials on the duration of stagnation in the areas of distribution of the oxygen deficiency in bottom layers, the data cited allowed establishment of the fact that desorption of phosphates, ammonium nitrogen, and silicic acid from the sediment in the bottom layer of the Azov Sea consisted of an average of 1.5, 28, and 35 thousand tons/year, respectively, for the period investigated (1970-77).

The values cited consist of approximately 27 and 20% of the total intake of nitrogen and phosphorus compounds from other external sources (river drainage, precipitation, conversion of shores, exchange with the Black Sea), and thus objectively demonstrate the exclusive role of desorption processes in formation of the chemical bases of marine productivity. Characteristically, the amount of desorbed nitrogen and phosphorus for the period analyzed consisted of approximatly 50% of their discharge with river water, and drainage basins with an area greater than 500,000 km^2 on the average.

The significant role of exchange processes in the biogenic balance of the Azov Sea is not a regional exception. In particular, according to the calculations of Yurkovskiy (1973), the amount of mineral phosphorus desorbed from sediment consisted of more than 58,000 tons only in the Silurian basin of the Baltic Sea for the stagnation period of 1931-33 and 1935-59. Yurkovskiy (1973), Fonselius (1972), and Sjoberg et al. (1972) believe that diffusion of phosphates from bottom sediments is a factor which enriches the entire aqueous layer of the Baltic Sea.

Based on the above, it is possible to speak of the difference in the chemical-biological reactions of the benthoic and pelagic regions of the sea in pollution of the "water-bottom" contact zone.

In the first case, the reaction takes place according to the scheme--accumulation of pollutants, increase in BOC, depletion of oxygen reserve, and formation of anaerobic or similar situations. The negative effects of this situation for the biocenosis in the contact zone are obvious and require no commentary.

In the second case, a decrease in the oxidative-reducing potential of the contact zone, up to the onset of reducing conditions, sharply activate desorption of biogenic elements in the surface layer of the sediment and enrichment of the bottom and overlying layers of the sea. The last circumstance is certainly a factor which to some degree stimulates processes of primary production of organic matter for the organic life and the pelagic zone in general. In contrast to the benthoic zone of the sea, the possibility of partial, total, or excessive compensation of the negative production effect of the toxic influence of pollutants is also totally real in this case.

This interpretation of the facts presented in the report is still inadequately supported by a system of empirical evidence. It is still to some degree a hypothesis whose development and refinement would obviously be facilitated by a more objective understanding of the complex biogenocenological consequences of pollution of the sea.

REFERENCES

Aleksandrova, Z. V., and A. M. Bronfman. 1975. Exchange of biogenic elements in the "water-sediment" system and its role in formation of the chemical bases of marine productivity. Okeanologiya 15(1).

Aleksandrova, Z. V., and M. G. Romova. 1977. The role of sediment in the formation of bottom oxygen deficiency. In The Biogeography of the Azov Sea and Its Basin. Leningrad.

Ayvazova, L. Ye., and M. V. Fedosov. 1972. The solubility of phosphates in sea water. In Studies in Theoretical and Applied Marine Chemistry. Nauka Press, Moscow.

Bronfman, A. M. 1976. Alternative solutions in the economic-ecologic problems of the Azov Sea basin. In Problems of Marine Economics. No. 5, Odessa.

Bronfman, A. M., and A. B. Gorstko. 1978. Quantitative evaluation of some chemical-biological consequences of pollution of the sea by petroleum products and detergents. Eokologiya No. 2.

Fillos, G., and H. Biswas. 1976. Phosphates release and sorption by Lake Mohegan sediment. J. Environ. Eng. Div. Proc. Amer. Soc. Civ. Eng. 102(2).

Fonselius, S. H. 1972. On biogenic elements and organic matter in the Baltic. Ambio Spec. Rep. No. 1.

Hatchinson, D. 1969. Limnology. Progress Press, Moscow.

Lars, K. N. 1974. Mud-water exchange of phosphate and other ions in undisturbed sediment cores and factors affecting the exchange rates. Arch. Hydrobiol. No. 2.

Lech, K. 1977. The mechanism of phosphate buffering in the water-bottom sediment system. Pol. Arch. Gidrobiol. 24(2).

Martynova, M. V. 1973. The problem of diffusion migration of dissolved forms of nitrogen from sludge in the bottom layers of water. In Interaction of Surface and Subterranean Effluents. Moscow University Publ. House, Moscow.

Oertzen, J. A. von. 1972. Marine pollution: a problem for marine biology. Biol. Rdsch.

Pirogova, M. V. 1953. Chemical exchange between the bottom and water layer in the Black Sea. Gidrokhimicheskiye materialy, Vol. 31.

Rowe, G. T., C. H. Clifford, K. L. Smith, and R. L. Hamilton. 1975. Benthic nutrient regeneration and its coupling to primary productivity in coastal waters. Nature 255(5505).

Shippel, F. A., and R. O. Hallberg. 1973. (Illegible) phosphate exchange at the sediment-water interface. Oikos 24, suppl. 15.

Sjoberg, S., F. Wulff, and P. Wahlstrom. 1972. The use of computer simulations for systems ecological studies in the Baltic. Ambio 1(6).

Vernadskiy, V. M. 1960. Biosphere. Selected studies. Vol. 5, Akad. Nauka SSSR Press, Moscow.

Yurkovskiy, A. K. 1972. The role of iron humate coagulants in accumulation of phosphates in the death of stagnation basins in the Baltic Sea. In Studies in Theoretical and Applied Marine Chemistry, Nauka Press, Moscow.

Yurkovskiy, A. K. 1973. The significance of some chemical processes in the benthoic layer of deep water basins in the dynamics of mineral phosphorus reserves in the Baltic Sea. In Fish Breeding Studies in the Baltic Sea Basin, No. 10, Zvaygzne Press, Riga.

☆USGPO: 1982 — 559-092/3415